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DATA EVALUATION RECORD

STUDY TYPE: One-Generation Reproduction Toxicity Study in Rats (gavage) OCSPP No Guideline; OECD 443.

PC CODE: 080800
TXR#: 0058011

DP BARCODE: D456901
SUBMISSION: 1025212

TEST MATERIAL (PURITY): BAS 850 H/Trifludimoxazin (98.5%)

SYNONYMS: Trifludimoxazin, 1,5-dimethyl-6-thioxo-3-[2,2,7-trifluoro-3-oxo-4-(prop-2-yn-1-yl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-1,3,5-triazine-2,4-dione

CITATION: Schneider, S., Strauss, V., Huisinga, M., Marxfeld, H., Grauert, E., van Ravenzwaay, B. (2018). BAS 850 H - Modified extended one-generation reproduction toxicity study in Wistar rats - Administration via the diet. BASF SE, 67056 Ludwigshafen, Germany. Laboratory Project ID.: 03R0343/09R123, 412179, March 22, 2018. MRID 50406139. Unpublished.

SPONSOR: BASF

EXECUTIVE SUMMARY: In a modified extended one-generational study (EOGRS) (MRID 50406139), BAS 850 H (98.5% ai; Batch COD-001645) was administered orally to groups of 30 male and 30 female healthy young Wistar rats as a homogeneous addition to the food at mean concentrations of 0, 75, 250, and 750 ppm; equivalent to 0, 6.4/6.7, 21.5/22.8, and 64.4/68.1 mg/kg bw/day (M/F). In order to maintain the compound intake of lactating dams, BAS 850 H dietary inclusion levels were reduced in females to 50% during the lactation of F1 and F2 litters due to increased food consumption. F0 animals were treated at least for 10 weeks prior to mating to produce a litter (F1 generation). Pups of the F1 litter were selected (F1 rearing animals) and assigned to 5 different cohorts which that were subjected to specific post-weaning examinations. Cohort 1B (=F1 generation parental animals) were selected to produce F2 pups. Groups of 25 male and 25 female F1 animals selected for breeding, continued to receive the same dose as their parents, and the breeding program was repeated to produce a F2 litter. The study was terminated with the terminal sacrifice of the F2 weanlings and F1 parental animals. Test diets containing BAS 850 H were offered continuously throughout the study. The Cohort 1A consisted of groups of 20 males and 20 females selected F1 weanlings treated for 10 weeks for the investigation of clinical biochemistry parameters, thyroid hormone levels in adult animals, determination of splenic subpopulations in the blood in the course of the immunotoxicological assessment and the determination of differential ovarian follicle counts. The 2A (adults) and 2B (weanlings) cohorts consisting of groups of 10 male and 10 female selected F1 weanlings served for the investigation

of neurotoxicity parameters, while groups of likewise 10 male and 10 female selected F1 weanlings served as Cohort 3 for the investigation of the T-cell dependent immune response in sheep red blood cells (SRBC) immunized rats. The sexual maturation of males and females F1 offspring was determined in all Cohort 1A, Cohort 1B, Cohort 2A and Cohort 3 animals.

Repeated analyses of BAS 850 H containing diets confirmed the stability, homogeneity and the intended concentration levels. No treatment-related mortality or clinical signs of toxicity were observed in adults or pups. The liver and thyroid were identified as target organs. Liver effects observed at the high dose included increased organ weight, increased GGT, and hypertrophy. Thyroid effects observed at the mid- and high-dose included increased incidence and severity of follicular cell hypertrophy/hyperplasia and altered colloid.

The LOAEL for parental/systemic toxicity is 250 ppm (equivalent to 21.5 and 22.8 mg/kg bw/d for males and females, respectively) based on increased incidence and severity of follicular cell hypertrophy/hyperplasia and altered colloid of the thyroid in males.

The reproductive performance of F0 and F1 (=Cohort 1B) male parental animals was affected by treatment with an increase of abnormal sperm observed at the high dose. No effects on the estrus cycle, mating behavior and fertility and the ability of females to deliver and rear their offspring was noted in either generation. Sporadically statistically significant differences of parameters were noted, but these were either not dose dependent, not observed in the other generation and generally within the historical control range.

The LOAEL for fertility and reproductive performance for the F0 and F1 male parental rats is 750 ppm (equivalent to 64.4 mg/kg bw/d for males and females, respectively) based on increased abnormal sperm. The NOAEL is 250 ppm (equivalent to 21.5 mg/kg/d).

The number, survival, body weight development and sex ratio of pups was not affected by treatment. Sexual maturation of males and females was comparable between all groups. No increase in the incidence of pup necropsy observations was noted with the exception of an increased incidence of dilated renal pelvises at the high dose of 750 ppm. However, dilated renal pelvises are a common finding in rats of the strain used. In the affected pups no compression of the parenchyma surrounding the renal pelvis was observed, which would have been an indication for an obstructed urogenital tract. The most likely underlying mechanism of renal pelvis dilation is the transient inhibition of renal growth, particularly regarding the length of the renal papilla during late gestation. Literature indicates that this type of renal pelvis dilation often is reversible postnatally (Woo and Hoar, 1972). Therefore, this finding is considered to be of no toxicological relevance.

The LOAEL for offspring toxicity cannot be established. The NOAEL is 750 ppm (equivalent to 64.4 and 68.1 mg/kg bw/d for males and females, respectively).

No neurotoxicological effects of treatment were recorded as neither the Functional Observation Battery (FOB) investigations (including home cage and open field observation, sensory motor and reflex tests and motor activity determination) in Cohort 2A animals at PND 72 nor the brain weight and/or brain morphology investigations at PND 22 (Cohort 2B) and PND 77 (Cohort 2A)

revealed any treatment-related changes. Furthermore, neither auditory startle response habituation at PND 24 nor neurohistopathological examination of Cohort 2A animals revealed effects related to treatment.

The LOAEL for developmental neurotoxicity for the F1 progeny cannot be established. The NOAEL is 750 ppm (equivalent to 64.4 and 68.1 mg/kg bw/d for males and females, respectively), the highest dose tested.

Investigation of the humoral, T-cell dependent immune response in sheep red blood cell (SRBC) immunized Cohort 3 rats did not reveal a change of Anti SRBC-IgM titers in blood six days after immunization. In contrast, Anti SRBC-IgM titers the Cyclophosphamide treated positive control animals were significantly decreased. The determination of splenic lymphocyte subpopulations (B and T lymphocytes, CD4+ and CD8+ lymphocytes and Natural killer cells) in Cohort 1A animals did not indicate an effect on cellular immune response.

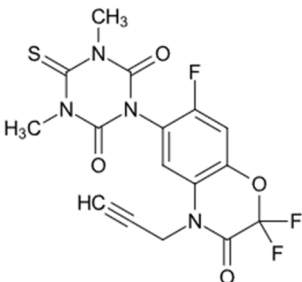
The LOAEL for developmental immunotoxicity for the F1 progeny cannot be established. The NOAEL is 750 ppm (equivalent to 64.4 and 68.1 mg/kg bw/d for males and females, respectively), the highest dose tested.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OCSPP No guideline; OECD 443) for an extended one-generation reproduction toxicity study in the rat.

COMPLIANCE: Signed and dated No Claim of Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** BAS 850 H (Reg No. 5654329)
Description: Solid/ beige
Lot/batch #: COD-001645
Purity: 98.5% a.i.
Compound stability: It was stated that the test substance was stable over the study period as indicated by the expiration date of April 1, 2015. Stability of the test item preparations in feed was analytically verified for a period of 32 days at room temperature before study start.
CAS # of TGAI: 1258836-72-4
Structure:

CN1C(=O)N(C)C(=O)N1C2=CC=C(C=C2OC3C(F)(F)CC3)CC#C
2. **Vehicle and/or positive control:** Vehicle: rodent feed. Positive control for developmental immunotoxicity: Cyclophosphamide Monohydrate (CPA) – Batch No. SLBC0666V; analytical purity: 102.3%; stability: stable over the study period; vehicle: drinking water; solid/white. Prior to the commencement of the study, the stability of the positive control substance CPA in drinking water at room temperature was analytically verified for a period of 7 days and for a period of 32 days when stored in freezer.
3. **Test animals:**
Species: Rat
Strain: CrI:WI (Han)
Age at study initiation: 36±1 day at dosing
Wt. at study initiation: Males: 108.2-140.8 g; Females: 94.9-122.5vg
Source: Charles River Laboratories, Research Models and Services, Germany GmbH
Housing: Rats were individually housed in Polycarbonate cages type III cages, with the following exceptions:
During overnight matings, male and female mating partners were housed together in Polycarbonate cages type III.
Pregnant animals and their litters were housed together until PND 21 (end of lactation).
Pregnant females were provided with nesting material (cellulose wadding) towards the end of gestation.
Dust-free wooden bedding was used, and for enrichment, wooden gnawing blocks
Diet: Kliba maintenance diet mouse/rat “GLP” meal (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*
Water: Drinking water, *ad libitum*
Environmental conditions: **Temperature:** 22±2°C
Humidity: 50±20%
Air changes: 15/h
Photoperiod: 12 h light/12 h dark
Acclimation period: Approximately 8 days

B. PROCEDURES AND STUDY DESIGN

1. **In-life dates:** Start: July 17, 2013 End: March 1, 2014
2. **Mating procedure:** Males and females from the same dose group were mated, overnight at a ratio of 1:1 for a maximum of 2 weeks. A vaginal smear was prepared each morning and examined for the presence of sperm. If sperm was detected, pairing of the animals was discontinued. The day on which sperm were detected was denoted "gestation day (GD) 0" and the following day "gestation day (GD) 1".
2. **Study schedule:** The P generation males and females were dosed for a minimum of 10 weeks prior to mating. F₀/F₁ females were allowed to deliver and rear their pups (F₁/F₂ generation pups) until PND 21 (F₁ pups assigned to cohorts 1-3 and F₂ pups) or 22 (surplus F₁ pups sacrificed for gross examination and hormone analysis).

On PND 4, the individual litters were standardized in such a way that, where possible, each litter contained 5 male and 5 female pups (always the first 5 pups/sex and litter were taken for further rearing). If individual litters did not have 5 pups/sex, the litters were processed in such a way that the most evenly distributed 10 pups per litter were present for further rearing (e.g., 6 male and 4 female pups). Standardization of litters was not performed in litters with ≤ 10 pups.

3. **Animal assignment:** Animal assignments are presented in Tables 1a, b, and c and Figure 1. Before weaning of the F₁ generation pups on PND 21, 75 male and 75 females per group were randomly selected to be placed into cohorts according to the experimental procedure scheme detailed in Figure 1. Obvious runts (those pups whose body weight was $\frac{3}{4}$ of or 25% below the mean body weight of the control group, separate for sexes) were not included. For cohort 1A and 1B, one male **and** one female pup were selected from each litter. For all other cohorts, one male **or** one female pup were selected from the same litter. In addition, 10 male and 10 female F₁ surplus pups were selected for the immunotoxicity cohort positive control group.

TABLE 1a. Animal assignment ^a						
Test group	ppm	Mean Dose (mg/kg/day) (M/F)	Animals/group			
			F0 Males	F0 Females	F1 Males ^b	F1 Females ^c
Control	0	0	30	30	75	75
Low	75	6.4/6.7	30	30	75	75
Mid	250	22/23	30	30	75	75
High	750	64/68	30	30	75	75

^a Data were obtained from pages 62-64 and page 114 of the study report.

F₁ generation rats assigned to Cohorts 1A, 1B, 2A, 2B and 3.

TABLE 1b. Offspring (F1) cohort assignment ^a											
Test group	Dose (mg/kg/day) (M/F)	Animals/group									
		Cohort 1A (Reproductive)		Cohort 1B (Reproductive)		Cohort 2A (Neurotoxicity)		Cohort 2B (Neurotoxicity)		Cohort 3 (Immunotoxicity)	
		Males	Females	Males	Females	Males	Females	Males	Females	Males	Female
Control	0	20	20	25	25	10	10	10	10	10	10
Low	6.4/6.7	20	20	25	25	10	10	10	10	10	10
Mid	22/23	20	20	25	25	10	10	10	10	10	10
High	64/68	20	20	25	25	10	10	10	10	10	10

^a Data were obtained from pages 63-64 of the study report.

TABLE 1c. Positive control group for immunotoxicity study ^a					
Test group	Dose (mg/kg/day)	Concentration (mg/100 mL)	Volume (mL/kg bw/d)	Males	Females
Positive Control	4.5	45	10	10	10

^a Data were obtained from page 59 of study report

Figure 1: Experimental procedure scheme^a

Dosing				
	Pre-mating	Mating	Post-mating	
F0 males	10 weeks	2 weeks	6 weeks	
F0 females	10 weeks	2 weeks	Pregnancy	Lactation
		F ₁	In-utero development	Pre-weaning
Post-weaning				

Parental generation	Cohort	Designation	Animals/Cohort	Puberty	Approximal age at necropsy (weeks)
Target is 25 litters per group	1A	Reproductive	20 M + 20 F	Yes	13
	1B	Reproductive	25 M + 25 F	Yes	20-25 (see scheme below)
	2A	Neurotoxicity	10 M + 10 F #	Yes	11
	2B	Neurotoxicity	10 M + 10 F #	No	3
	3	Immunotoxicity	10 M + 10 F #	Yes	8
	Surplus	Spares		No	3

one per litter and representative of 20 litters in total where possible

Dosing				
	Post-weaning / Pre-mating	Mating	Post-mating	
Cohort 1B males	10 weeks	2 weeks	6 weeks	
Cohort 1B females	10 weeks	2 weeks	Pregnancy	Lactation
		F ₂	In-utero development	Pre-weaning

^a Obtained from page 64 of study report

4. **Dose selection rationale:** In a previous one-generation range finding study (17R0343/09R084) groups of 10 male and female rats were administered BAS 850 H at dietary dose levels of 0, 300, 650, 1250 and 2500 ppm. Six weeks after commencement of treatment male and female rats were mated on a 1:1 ratio to produce offspring. The females were allowed to deliver and rear their F1 pups until PND 4 (standardization) or PND 21 (weaning). After weaning all F0 parental animals and F1 pups were sacrificed.

Findings relevant for dose selection consisted of excessive toxicity at 2500 ppm, such as death, severe clinical signs (gait changes, apathia, tremors and lateral position), markedly reduced food consumption and body weight loss. The 2500 ppm dose level was terminated

after 9 days for humane reasons. At 1250 ppm, only 2/10 males induced pregnancy in females. Sperm analysis revealed a substantial decrease of sperm motility to a mean of 11% and an increase of abnormal sperm to a mean of 83%. While the number of homogenization resistant testicular sperm heads was not affected, the number of sperm heads in the cauda epididymis was significantly reduced by about 38%. No effects on fertility were observed at dose levels ≤ 650 ppm. A special range-finding study investigating the effects on epididymal sperm in young male rats, performed at 0, 750, 1000 and 1250 ppm, revealed a substantial decrease of motility ($\leq 50\%$ motile sperm) as well as an increase of abnormal sperm heads ($\geq 40.5\%$) at 1000 ppm and above, while there were only minor effects at 750 ppm when compared to the concurrent control (See Appendix I for more information).

- 5. Dosage preparation and analysis:** The required quantity of test substance was weighed in a beaker depending on the dose group and thoroughly mixed with a small amount of food. Then, further amounts of food were added to this premix as needed and finally mixed for about 10 minutes in a laboratory mixer. Diet preparations were analyzed for homogeneity and test substance content.

After the acclimatization period, the test substance was administered to the parental animals as addition to the diet continuously throughout the entire study. The animals of the control group were treated in the same way, with the vehicle (diet only). Treatment ended about 16 hours before sacrifice.

Concentration control analyses were carried out at the beginning and towards the end of the F_0 premating phase, as well as for female diets once in the F_1/F_2 lactation periods. Homogeneity investigations were conducted in diets at the beginning of the pre-mating period and once in each lactation period with the lowest and highest concentrations in each case.

With one exception, all mean values for BAS 850 H were in the expected range of the target concentrations (90-110%), demonstrating the correctness of the diet preparations. The exception was one lactational low-dose sample which was slightly above (113.7%) the specification limit of 110%, however, mean concentrations within $\pm 15\%$ of the target concentration can be regarded acceptable for complex matrices like diet.

In general, homogeneity of the diet preparations was verified analytically as demonstrated by low relative standard deviations of $\leq 5\%$. Albeit the second lactational samples at the low and high dose revealed a relative standard deviation slightly above 5% (7.4% and 6.2% for the low and high dose, respectively), however, in general, relative standard deviations $\leq 10\%$ can be regarded as acceptable for diet analysis.

Results:

Homogeneity analysis (%RSD): 0.3-7.4%

Stability analysis: The stability of the test substance in rat diet was demonstrated for a period of 32 days at room temperature.

Concentration analysis (% nominal; mean): 92.1-113.7%

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. **Dosage administration:** After the acclimatization period, the test substance was administered to the parental animals as addition to the diet continuously throughout the entire study. The animals of the control group were treated in the same way, with the vehicle (diet only). Treatment ended about 16 hours before sacrifice.

F1 animals were treated with the test substance at the same dose level as their parents, from post-weaning through adulthood.

During lactation period, the BAS 850 H concentration in the diet of the parental females was reduced to 50%, resulting in 0, 37.5, 125, 375 ppm (this dietary adjustment derived from historical body weight and food consumption data maintained the dams at the desired target doses of BAS 850 H during this period of increased food intake). Reducing the feed concentration during lactation maintained a constant dose.

C. **OBSERVATIONS**

1. **Parental animals:** Each animal was examined for mortality or moribund condition at least twice daily on working days and once daily on weekends and public holidays. Moribund animals were sacrificed and necropsied. Cage-side clinical signs observation (morbidity, behavioral changes, overt toxicity) were recorded at least once a day during the study period. Any abnormalities were recorded with the date of onset, nature, degree, and duration. Estrous cycle length was evaluated by daily analysis of vaginal smear for all F0 and F1 (cohort 1B) parental female rats for a minimum of 3 weeks prior to mating.

The parturition and lactation behavior of the dams was generally evaluated in the mornings in combination with the daily clinical inspection of the dams. On weekdays, parturition behavior was additionally inspected in the afternoons. The day of parturition was considered the 24-hour period from about 15 h of one day until about 15 h of the following day.

Detailed clinical observations were performed in the mornings for all F₀ parental and F₁ animals and in cohorts 1A, 1B, 2A and 3 at weekly intervals during the administration period. The findings were ranked according to the degree of severity, if applicable. For observation, the animals were removed from their cages by the investigator and placed in a standard arena (50 × 37.5 × 25 cm). The following parameters were assessed: 1) abnormal behavior in handling; 2) fur; 3) skin; 4) posture; 5) salivation; 6) respiration; 7) activity/arousal level; 8) tremors; 9) convulsions; 10) abnormal movements; 11) gait abnormalities; 12) lacrimation; 13) palpebral closure; 14) exophthalmos (protruding eyeball); 15) assessment of feces excreted during the examination (appearance/consistency); 16) assessment of the urine excreted during the examination; and 17) pupil size.

2. **Clinical pathology:** Blood and urine samples were drawn from F₀ parental and F₁ cohort 1A, 10 males and 10 females per group, at termination. Blood samples were taken from animals by puncturing the retrobulbar venous plexus following isoflurane anesthesia. Blood sampling and blood examinations were carried out in a randomized sequence. In the afternoon preceding the day of urinalysis, the animals were individually transferred into metabolism cages (no food or drinking water provided); on the following morning, the individual urine specimens were examined in a randomized sequence. Thyroid hormones TSH and T₄ were assessed in the morning between 8:00 and 10:00 am for F₀, F₁ surplus, PND22, F₁ Cohort 3, and F₁ Cohort 1A.
- a. **Hematology:** The following parameters were determined in blood with EDTA-K₃ as anticoagulant using a particle counter, except clotting tests were carried out using a ball coagulometer:

Hematology:		
<i>Red blood cells</i>	<i>White blood cells</i>	<i>Clotting Potential</i>
✓ Erythrocyte count (RBC)	✓ Total leukocyte count (WBC)	✓ Prothrombin time (HQT)
✓ Hemoglobin (HBG)	✓ Neutrophils (differential)	✓ Platelet count
✓ Hematocrit (HCT)	✓ Eosinophils (differential)	Partial thromboplastin time
✓ Mean corp. volume (MCV)	✓ Basophils (differential)	✓ Thromboplastin time (i.e. prothrombin time)
✓ Mean corp. hemoglobin (MCH)	✓ Lymphocytes (differential)	
✓ Mean corp. Hb. conc. (MCHC)	✓ Monocytes (differential)	
✓ Reticulocytes	✓ Large unstained cells (differential)	

b. **Clinical chemistry:**

Clinical chemistry:		
<i>Electrolytes</i>	<i>Metabolites and Proteins</i>	<i>Enzymes:</i>
✓ Calcium	✓ Albumin	✓ Alanine aminotransferase (ALT)
✓ Chloride	✓ Bilirubin (total)	✓ Aspartate aminotransferase (AST)
✓ Magnesium	✓ Cholesterol	✓ Alkaline phosphatase (ALP)
✓ Phosphorus (inorganic)	✓ Creatinine	✓ γ-Glutamyl transferase (GGT)
✓ Potassium	✓ Globulin (by calculation)	
✓ Sodium	✓ Glucose	
	✓ Protein (total)	
	✓ Triglycerides	
	✓ Urea	

- c. **Urinalysis:** Dry chemical reaction on test strips and a reflection photometer were used for the semiquantitative evaluation of the following, except sediment, color/turbidity and volume (evaluated by microscopy, visually and graduated tube, respectively):

Urinalysis		
<i>Quantitative parameters:</i>	<i>Semi-quantitative parameters</i>	
✓ Urine volume	✓ Bilirubin	✓ Protein
✓ Specific gravity	✓ Blood	✓ pH-value
	✓ Color and turbidity (visual exam.)	✓ Urobilinogen
	✓ Glucose	✓ Sediment (microscopic exam.)
	✓ Ketones	Nitrite

- d. **Hormones:** Calibration curves for the following assays were provided in a separate report (MRID 50807502).

Hormone Parameter	Unit	LOD ^a	LLOQ ^b	ULOQ	Lowest standard	Method
Total thyroxine (T4)	nmol/L	10	25		25	ELISA
Thyroid stimulating hormone (TSH)	µg/L	0.5	4		1	Direct, competitive radioimmunoassay

^a Limit of detection according to commercial kit manufacturer's instructions.

^b Lower limit of quantification according to laboratory validation using rat serum samples determined by the Agency.

3. Litter observations

- a. **Pup number and status at delivery:** All pups delivered from the F₀ parents (F₁ litter) and the F₁ parents (F₂ litter) were examined as soon as possible on the day of birth to determine the total number of pups, the sex and the number of liveborn and stillborn pups in each litter. At the same time, the pups were also being examined for macroscopically evident changes. Pups which died before this initial examination were defined as stillborn pups.
- b. **Pup viability/mortality:** In general, a check was made for any dead or moribund pups twice daily on workdays (once in the morning and once in the afternoon) or as a rule, only in the morning on Saturdays, Sundays or public holidays. Dead pups were evaluated by necropsy.

The number and percentage of dead pups on the day of birth (PND 0) and of pups dying between PND 1-4, 5-7, 8-14 and 15-21 (lactation period) were determined; however, pups, which died prior to termination date or had to be sacrificed due to maternal death, were not included in these calculations. The number of live pups/litter was calculated on the day after birth, and on lactation days 4, 7, 14, and 21. Furthermore, viability and lactation indices were calculated according to the following formulas:

$$\text{Viability index [\%]} = \frac{\text{number of live pups on day 4* after birth}}{\text{number of live pups on the day of birth}} \times 100$$

* before standardization of litters (i.e. before culling)

$$\text{Lactation index [\%]} = \frac{\text{number of live pups on day 21 after birth}}{\text{number of live pups on day 4* after birth}} \times 100$$

* after standardization of litters (i.e. after culling)

- c. **Sex ratio:** On the day of birth (PND 0), the sex of the pups was determined by observing the distance between the anus and the base of the genital tubercle; normally, the anogenital distance is considerably greater in male than in female pups. Later, during the course of

lactation, this initial sex determination was followed up by surveying the external appearance of the anogenital region and the mammary line. The sex of the pups was finally confirmed at necropsy. The sex ratio was calculated at PND 0 and PND 21 according to the following formula:

$$\text{Sex ratio [\%]} = \frac{\text{number of live male or female pups on day 0 / 21}}{\text{number of live male and female pups on day 0 / 21}} \times 100$$

- d. **Pup clinical observations:** The live pups in cohorts 1A, 1B, 2A, and 3 were examined daily in the morning for clinical symptoms (including gross morphological findings) during the clinical inspection of the dams. Pups showing particular findings were documented along with the dam associated with the pup.
- e. **Nipple/areola anlagen:** All surviving male pups were examined for the presence of nipple/areola anlagen on PND 12 and were re-examined on PND 20 before necropsy.
- f. **Pup body weight data:** The pups were weighed on the day after birth (PND 1) and on PND 4 (before standardization), 7, 14 and 21. Body weight change was calculated from these results.
- g. **Anogenital distance:** Anogenital distance (AGD) was determined in all live male and female pups on PND 1. These measurements were performed in randomized order, using a measuring ocular. They were conducted by technicians unaware of treatment group in order to minimize bias. The anogenital index was calculated according to the following formula:

$\text{anogenital index} = \frac{\text{anogenital distance [mm]}}{\text{cubic root of pup weight [g]}}$

h. Sexual Development:

- i. **Vaginal opening:** All female F₁ pups selected to become the F₁ parental generation females (25/group; cohort 1B) and F₁ rearing animals (F₁ cohorts 1A, 2A, and 3) giving a total of 65 females/group were evaluated daily for vaginal opening beginning on PND 27. On the day of vaginal opening, the body weights of the respective animals were determined. Vaginal smears were collected after vaginal opening until the first cornified smear (estrous) was recorded.
- ii. **Balanopreputial separation:** All male F₁ pups selected to become the F₁ parental generation males (25/group; F₁ cohort 1B) and F₁ rearing animals (F₁ cohorts 1A, 2A, and 3) giving a total of 65 males/group were evaluated daily for balanopreputial separation beginning on PND 38. On the day of balanopreputial separation, the body weights of the respective animals were determined.
- i. **Pup organ weights:** After the scheduled sacrifice, the brain, spleen and thymus of the first male and the first female pups/litter from the F₂ pups were weighed. The corresponding in-life pup weights determined on PND 21 were used to calculate the relative organ weights.

- j. **Pup necropsy observations:** On PND 4, 10 culled F₁ pups/sex/group were sacrificed by decapitation under isoflurane anesthesia and blood was sampled for determination of serum thyroid hormone concentrations (TSH and T₄).

After a similar standardization on PND 4, the surplus F₂ pups were sacrificed under isoflurane anesthesia with CO₂. After sacrifice, these pups were examined externally, eviscerated and their organs were assessed macroscopically.

On PND 22, the surplus F₁ generation pups that were not used for the cohorts or any investigations were sacrificed under isoflurane anesthesia with CO₂ and were examined pathologically. A subset of 10 surplus F₁ pups/sex/group selected for hormone analyses were sacrificed by decapitation under isoflurane anesthesia in the pathology lab and blood was sampled for thyroid hormone analyses (TSH and T₄).

On PND 21, all F₂ generation pups were sacrificed under isoflurane anesthesia with CO₂. After sacrifice, these pups were examined externally, eviscerated and their organs were assessed macroscopically. Microscopic examinations were not conducted on the pups.

The brain, spleen and thymus were weighed in one surplus F₂ weanling per sex per litter (as a rule the first available male and female pup per litter). The relative organ weights were calculated from these weights and the weight of the selected living pup at sacrifice.

- k. **Clinical pathology:** Blood samples for thyroid hormone evaluation were collected from 10 surplus (culled) PND 4 offspring and 10 surplus PND 22 offspring *via* decapitation (following isoflurane anesthesia). PND 4 samples only were pooled per sex and litter if the available amount was not sufficient for a hormone analysis. The same hematology, clinical chemistry, urinalysis, and thyroid hormone parameters as the P rats were examined.

l. **Behavioral testing (Cohort 2A)**

- i. **Acoustic Startle Habituation:** Acoustic startle habituation was evaluated on PND 24 using the SR-LAB; STARTLE RESPONSE SYSTEM (San Diego Instruments, San Diego, CA, USA). The examinations were started in the morning with age-appropriate size enclosures. Rats were tested in a randomized sequence for their reactivity to auditory stimuli and habituation of responses with repeated presentation of stimuli. The test session consisted of a 5-minute adaptation period with a background noise of 70 decibels (dB). No data were collected during this period. Then the startle response was recorded in 50 trials at a startle stimulus sound level of 120 dBA with a 5 - 10 second variable interval between the trials. Response was recorded for 50 milliseconds. Measurement was carried out with the light and ventilator switched on in the measurement chambers; no food or water was provided during the test. Data (maximum amplitude, latency to the peak of the response) were analyzed in 5 blocks of 10 trials each. The San Diego Instruments SR ASR lab measures voltage changes during the response window. The maximum amplitude is the highest voltage during the response window (i.e., = peak of response). The voltages are then converted by the SR software into “units” based on (milli)volts.

- ii. **Functional observational battery (FOB):** The FOB was carried out once in all F₁ cohort 2A animals at PND 72. The FOB was carried out in a randomized sequence. The animals were not transferred to new cages before the test, nor was food or drinking water withdrawn. The FOB was started with passive observations without disturbing the rats, followed by removal from the home cage, open field observations in a standard arena and sensory motor tests as well as reflex tests. The findings were ranked according to their degree or severity, if applicable.

During the home cage observation, special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.

For open field observation, the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1. behavior when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements / stereotypes
5. nose discharge	14. gait abnormalities
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. feces (number of fecal pellets/appearance/consistency) within two minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1. reaction to an object being moved towards the face (approach response)	8. behavior during handling
2. touch sensitivity (touch response)	9. vocalization
3. vision (visual placing response)	10. pain perception (tail pinch)
4. pupillary reflex	11. grip strength of forelimbs
5. pinna reflex	12. grip strength of hind limbs
6. audition (startle response)	13. landing foot-splay test
7. coordination of movements (righting response)	14. other findings

- iii. **Motor activity:** Motor activity (MA) measurement was carried out in all animals of F₁ cohort 2A at PND 72. The MA was measured from 12 h onwards on the same day as the FOB was performed. The examinations were performed using the TSE Labmaster System supplied by TSE Systems GmbH, Bad Homburg, Germany. For this purpose, the animals were placed in clean polycarbonate cages with a small amount of bedding for the duration of the measurement. Eighteen beams were allocated per cage. The number of beam interrupts were counted over 12 intervals for 5 minutes per interval. The sequence at which the animals were placed in the cages was selected at random. The individual measurement period began when the 1st beam was interrupted and finishes exactly 1 hour later. No food or water was offered to the animals during these measurements. After the transfer of the last animal in each case, the measurement room was darkened.

4. Developmental immunotoxicity (Cohorts 1A and 3):

- a. **T-cell dependent antibody response:** All F₁ cohort 3 males and females and 10 positive control animals were immunized by intraperitoneal injection of 0.5 ml (in two 0.25 ml portions at one occasion) of a 4×10^8 SRBC/ml suspension (SRBC = Sheep Red Blood Cell). Six days after immunization, blood was withdrawn from the retrobulbar venous plexus under isoflurane anesthesia from all males and females of F₁ cohort 3. The samples were used to assess the functional responsiveness of major components of the immune system to a T-cell dependent antigen, sheep red blood cells (SRBC) on PND 59. For this purpose, an anti SRBC-IgM determination was performed by ELISA.
- b. **Splenic lymphocyte subpopulation analysis:** All males and females of F₁ cohort 1A (terminated on PND 90) were used to determine splenic lymphocyte subpopulations using one half of the spleen (the other half was used for pathology examination as indicated below). The immunophenotyping was performed with a flow cytometer after staining with cell specific antibodies. The absolute counts of the cell fractions were determined.

The following determinations were performed:

- B lymphocytes
- T lymphocytes
- CD4+ lymphocytes
- CD8+ lymphocytes
- Natural killer cells
- Ratio B/T lymphocytes
- Ratio CD4+/CD8+ lymphocytes

The B:T cell ratio as well as the CD4+:CD8+ cell ratio were calculated using the relative cell counts.

5. Postmortem observations

- a. **Parental animals:** All F₀ parental were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology, special attention being given to the reproductive organs. Immediately after necropsy and organ weight determination, the right testis and cauda epididymis were taken from all male F₀ parental animals. Sperm motility, sperm morphology, sperm head count (cauda epididymis), and sperm head count (testis) examinations were carried out. A vaginal smear was examined to determine the stage of estrous cycle for each F₀ and F₁ females.

After weaning of F₂ offspring, all F₁ parental (F₁ cohort 1B) animals were sacrificed by exsanguination. Animals were first subjected to isoflurane sedation, then deeply anesthetized by intraperitoneal injection of a mixture of pentobarbital and heparin.

The exsanguinated animals were necropsied and assessed by gross pathology, special attention being given to the reproductive organs.

potential future histopathological examination.

All F₁ and F₂ pups which were not used for other purposes without any notable findings were discarded after their macroscopic evaluation.

- ii. **Cohort 1:** Cohort 1A rats were euthanized at approximately PND 90. F₁ generation rats selected for clinical pathology evaluation and/or hormone analyses were euthanized by decapitation under isoflurane anesthesia. All males and females of Cohort 1A were used to determine splenic lymphocyte subpopulations using one half of the spleen. The absolute counts of the cell fractions were determined. Primordial and growing ovarian follicles were counted (individually for both ovaries in) in the control and top dose groups (F₁ cohort 1A females) according to definitions in Plowchalk et.al. (1993). To prevent multiple counting for growing follicles, only follicles with an oocyte with a visible nucleus were counted. Spleens of 10 animals per sex per group of Cohort 1A were split in two comparable parts (transversally). One part of the spleen was fixed in 4% buffered formaldehyde and afterwards embedded in paraplast. The other part of the spleen was used for immunophenotyping (as described above). Immediately after necropsy and organ weight determination, the right testis and cauda epididymis were taken from all male F₁ cohort 1A males. Sperm motility, sperm morphology, sperm head count (cauda epididymis), and sperm head count (testis) examinations were carried out.

F ₁ cohort 1A animals											
The following organs were collected (column C), weighed (W: ✓: all groups) and examined histopathologically (H: ✓: all groups, #: control and top dose animals, §: all affected animals; \$: all control and top dose animals and additionally all mid dose animals suspected of reduced fertility).											
C	W	H		C	W	H		C	W	H	
			adipose tissue	✓		#	jejunum (with Peyer's patches)	✓	✓	✓	seminal vesicles**
✓	✓	\$	adrenals	✓	✓	#	kidneys				skin
			aorta				lachrymal glands	✓		#	spinal cord [§]
✓		#	bone marrow (femur)	✓	✓	✓	liver	✓	✓	#	spleen
✓	✓	#	brain	✓		#	lungs				sternum w. marrow
✓		#	caecum	✓	✓	#	lymph nodes*	✓		#	stomach [§]
✓		#	colon	✓		#	mammary gland	✓	✓	✓	testes ^{##}
	✓		cauda epididymis	✓		#	muscle, skeletal	✓	✓	#	thymus
✓		#	duodenum	✓		#	nerve (sciatic)	✓	✓	✓	thyroid/ parathyroid glands ^{&}
✓	✓	✓	epididymides ^{##}	✓		#	esophagus				tongue
✓		#	eyes with optic nerve	✓	✓	✓	ovaries / oviduct [#]				tonsils
			femur (with knee joint)	✓		#	pancreas	✓		#	trachea
			gall bladder	✓	✓	✓	pituitary	✓		#	urinary bladder
✓		\$	gross lesions	✓	✓	✓	prostate	✓	✓	✓	uterus with cervix
✓	✓	#	heart	✓		#	rectum	✓		✓	vagina
			hind-limb (one)				salivary glands	✓		✓	vas deferens
✓		#	ileum				hind-limb (one)	✓			body
* mesenteric and axillary – weighed for F ₁ cohort 1A animals only; ** including coagulation glands; # oviduct not weighted, ovaries fixed in modified Davidson's solution; ## left epididymis and testis was fixed in modified Davidson's solution; § forestomach and glandular stomach; [§] cervical-, thoracic- and lumbar cord; & parathyroid glands were assessed histopathologically for control and top dose animals, only											

- iii. **Cohort 2:** On PND 22, F₁ cohort 2B animals were weighed, subjected to deep anesthesia (pentobarbital) and sacrificed by perfusion fixation. The length and maximum width of the brain was measured in all animals. The organs processed and details on the examination are given in the table below:

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Pathology:											
The following organs were collected (column C), weighed (W) and examined histopathologically* (H, ✓: all perfused groups, #: all affected perfused animals).											
C	W	H		C	W	H		C	W	H	
✓		#	all gross lesion				Spinal cord, cervical part (C1-C5)	✓		✓	Trigeminal ganglia
✓	✓	✓	brain with olfactory bulb*				Spinal cord, thoracic part (Th5-8)				Root fibers, dorsal (C1-C6 and L1-L4)
✓			eyes with retina and optic nerve				Spinal cord, lumbar part (L1-L4)				Root fibers, ventral (C1-C6 and L1-L4)
			M. gastrocnemius				Spinal ganglia (C1-C5 [3x])				
✓			nose (nasal cavity)				Spinal ganglia (L1-L4 [3x])				
✓	✓		pituitary gland				Tibial nerve (on the knee), proximal section				
			sciatic nerve, proximal section				Tibial nerve (nerve branch in the lower leg muscles), distal section				
* details of the histopathological examinations see below											
Brain cross sections							Brain associated organs/tissues				
Olfactory bulb							Pituitary gland				
Prosencephalon with frontal lobe							Trigeminal ganglia with part of nerve				
Diencephalon with parietal lobe											
Mesencephalon with occipital lobe and temporal lobe											
Pons											
Cerebellum (2 planes of section) ¹											
Medulla oblongata											
¹ The cerebellum was separated from the remaining brain in the cerebellar peduncle and divided into two halves. A cross section in the region of the midcerebellum was made in one half, and a longitudinal section was made through the vermis in the other half.											

On PND 77, all F₁ cohort 2A animals were weighed, subjected to deep anesthesia (pentobarbital) and sacrificed by perfusion fixation. The length and maximum width of the brain was measured in all animals. The organs processed and details on the examination are given in the table below:

Pathology:											
The following organs were collected (column C), weighed (W) and examined histopathologically* (H, ✓: all perfused groups, #: all affected perfused animals).											
C	W	H		C	W	H		C	W	H	
✓		#	all gross lesion	✓	✓		Spinal cord, cervical part (C1-C5)	✓	✓		Trigeminal ganglia
✓	✓	✓	brain with olfactory bulb	✓	✓		Spinal cord, thoracic part (Th5-8)	✓	✓		Root fibers, dorsal (C1-C6 and L1-L4)
✓		✓	eyes with retina and optic nerve	✓	✓		Spinal cord, lumbar part (L1-L4)	✓	✓		Root fibers, ventral (C1-C6 and L1-L4)
✓		✓	M. gastrocnemius	✓	✓		Spinal ganglia (C1-C5 [3x])				
✓		✓	nose (nasal cavity) with olfactory epithelium	✓	✓		Spinal ganglia (L1-L4 [3x])				
✓		✓	pituitary gland	✓	✓		Tibial nerve (on the knee), proximal section				
✓		✓	sciatic nerve, proximal section	✓	✓		Tibial nerve (nerve branch in the lower leg muscles), distal section				
For details of the histopathological examinations see below											

Pathology:	
The following organs were collected (column C), weighed (W) and examined histopathologically* (H, ✓: all perfused groups, #: all affected perfused animals).	
Brain (cross sections) Olfactory bulb Prosencephalon with frontal lobe Diencephalon with parietal lobe Mesencephalon with occipital lobe and temporal lobe Pons Cerebellum (2 planes of section) ¹ Medulla oblongata	Peripheral nervous system: Trigeminal ganglia with part of nerve M. gastrocnemius (longitudinal and cross sections) Dorsal root ganglia (3 out of C3-C6) Dorsal root fibers (C1-C6) Ventral root fibers (C1-C6) Dorsal root ganglia (3 out of L1-L4) Dorsal root fibers (L1-L4) Ventral root fibers (L1-L4) Proximal sciatic nerve (longitudinal and cross sections) Proximal tibial nerve (on the knee: longitudinal and cross sections) Distal tibial nerve (nerve branch in the lower leg muscles: longitudinal and cross sections)
Brain associated organs/tissues Eyes with retina and optic nerve Pituitary gland Olfactory epithelium (nose level III)	Spinal cord (longitudinal and cross sections): Cervical cord I (C1-C3) Cervical cord II (C3-C5) Thoracic cord (T5-T8) Lumbar cord (L1-L4)
¹ The cerebellum was separated from the remaining brain in the cerebellar peduncle and divided into two halves. A cross section in the region of the midcerebellum was made in one half, and a longitudinal section was made through the vermis in the other half.	

Thickness measurements of major brain layers (neocortex: frontal and parietal cortices, caudate nucleus/putamen, hippocampus, corpus callosum, cerebellum) were performed. Measurements were carried out bilaterally in the left and right halves of the brain with the exception of the corpus callosum and the cerebellum. For details of the selection of the planes and the conduct of the measurements see Page 103 of the study report.

iii. Cohort 3: These animals were used for the T-cell dependent antibody response test as indicated above. All F₁ cohort 3 animals were sacrificed by decapitation under isoflurane anesthesia. All animals were necropsied and assessed by gross pathology. Cadaver, spleen, thymus were weighed, and all gross lesions, spleen, thymus were fixed in 4% buffered formaldehyde. Neither histochemical processing nor histopathology was performed.

D. DATA ANALYSIS

1. Statistical analyses:

Parameter	Statistical test
Statistics of clinical examinations	
Food consumption ¹ , body weight and body weight change (parental animals and pups; for the pup weights, the litter means were used), estrous cycle duration, number of mating days, duration of gestation, number of implantation sites, post-implantation loss and % post-implantation loss, number of pups delivered per litter, duration of sexual maturation (days to vaginal opening, days to preputial separation), anogenital distance, anogenital index	Simultaneous comparison of all dose groups with the control group using the DUNNETT-test (two-sided) for the hypothesis of equal means
Male and female mating indices, male and female fertility indices, gestation index, females with liveborn pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups sacrificed moribund, viability index, lactation index, number of litters with affected pups at necropsy, sexual maturation data (vaginal opening, preputial separation)	Pairwise comparison of each dose group with the control group using FISHER'S EXACT test for the hypothesis of equal proportions
Presence of areolae/nipples, proportions of affected pups per litter with necropsy observations	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Rearing, grip strength of forelimbs and hindlimbs, landing foot-splay test, motor activity, startle response	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the equal medians
Statistics of clinical pathology	
Blood parameters and splenic lymphocytes subpopulations	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians
Urinalysis parameters (apart from pH, urine volume, specific gravity, color and turbidity)	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians. In case of exactly the same numbers of the dose group and the control, no statistical test was performed.
Urine pH, volume and specific gravity	Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians.
Urine color and turbidity	Urine color and turbidity are not evaluated statistically.
Sperm-analysis parameters	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) with Bonferroni-Holm adjustment for the hypothesis of equal medians. For the percentage of abnormal sperms values < 6% were set to 6% (cut off 6%).

1. Note: For the parameter food consumption, the "mean of means" was calculated and can be found in the relevant summary tables. The "mean of means" values allow a rough estimation of the total food consumption during the different time intervals (premating, gestation and/or lactation); they are not exactly precise values, because the size of the intervals taken for calculation may differ (especially during gestation and lactation periods). For the "mean of means" values no statistical analysis was performed.

Parameter	Statistical test
Statistics of pathology	
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS-test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using the WILCOXON-test (two-sided) for the equal medians. A pairwise comparison of the positive control group (F ₁ generation, rearing animals, cohort 3, Immunotoxicity) with the control group was performed using WILCOXON-test (two-sided) for the equal medians.
DOFC (differential ovarian follicular count)	Pair-wise comparison of the dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians.
Statistics of neurohistopathology	
Weight parameters (brain)	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the equal medians.
Brain width and length	Pair-wise comparison of control group with test groups using the WILCOXON-test (two-sided) with Bonferroni-Holm-Adjustment for the hypothesis of equal medians.
Brain morphometry: linear measurements of selected brain regions	Pair-wise comparison of placebo group with test groups using the WILCOXON-test (two-sided).

The statistical analyses were considered appropriate.

2. **Indices:**

Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

$$\text{Male/female fertility index} = \frac{\# \text{ pregnancies}}{\# \text{ of rats mated}}$$

$$\text{Male/female mating index} = \frac{\# \text{ females/males mated}}{\# \text{ of rats placed with opposite sex}}$$

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

$$\text{Gestation index} = \frac{\# \text{ rats with live offspring}}{\# \text{ pregnant rats}}$$

$$\text{Viability index} = \frac{\# \text{ live pups on PND 4 (preculling)}}{\# \text{ liveborn pups on PND 0}}$$

$$\text{Sex ratio (\%)} = \frac{\# \text{ live male or female pups on PND 0 /PND 21}}{\# \text{ live male or female pups on PND 0 /PND 21}}$$

3. **Historical control data:** Historical control data were provided for mean maternal body

weights during gestation and lactation periods, estrous cycle number and length, mating data, delivery data, litter data, pup weights, AG distance and AG index (PND1), pup organ weights, pup necropsy observations, sexual maturation data, auditory startle response maximum amplitude, auditory startle response latency to the peak, clinical pathology, sperm parameters, and F0 organ weights.

II. RESULTS

A. PARENTAL ANIMALS (P Generation)

1. Mortality and clinical signs

- a. **Mortality:** There were no treatment-related mortalities.
- b. **Clinical signs:** No clinical signs attributable to dose were observed. One incidental finding of skin lesion at week 8 in a control male was observed.

2. Body weights, body weight gains, and food consumption

- a. **Males:** There were no effects on male body weights, body weight gains, or food consumption at any dose level tested. (Table 4a).

Observation/study day	Dose group (mg/kg/day)			
	0	6.4	21.5	64.4
Body weight (g) Week 0	126.5 \pm 7.6	126.6 \pm 7.84	125.5 \pm 7.51	125.1 \pm 8.13
Week 2	209.3 \pm 12.23	213.4 \pm 11.63	211.9 \pm 10.39	211.6 \pm 14.02
Week 10	371.6 \pm 24.56	380.1 \pm 32.55	374.0 \pm 30.42	377.0 \pm 31.82
Week 15	401.1 \pm 31.28	414.8 \pm 38.00	404.5 \pm 33.07	404.1 \pm 34.48
Body weight gain (g) Week 0-15	276.6 \pm 28.55	288.1 \pm 35.56	278.9 \pm 31.98	279.1 \pm 30.88
Food consumption (g/rat/day) Week 0-10	20.9 \pm 1.15	21.2 \pm 1.14	21.3 \pm 10.7	21.3 \pm 1.09

a Data were obtained from Tables IA-IA-009, IA-010, IA-014 on pages 183, 187-188, and 192 of the study report.

b. Females

- i. **Premating:** There were no effects of treatment on body weights, body weight gains, or food consumption during premating (Table 4b).

Observation/study day	Dose group (mg/kg/day)			
	0	6.7	22.8	68.1
Body weight (g) Week 0	106.7 \pm 5.49	106.1 \pm 5.51	106.9 \pm 5.69	106.8 \pm 5.83
Week 2	148.1 \pm 7.47	148.4 \pm 8.22	150.6 \pm 9.56	152.3 \pm 9.02
Week 10	217.5 \pm 13.19	217.8 \pm 15.85	219.1 \pm 17.72	219.5 \pm 17.36
Body weight gain (g) Week 0-10	110.8 \pm 12.89	111.7 \pm 14.60	112.2 \pm 16.58	112.8 \pm 15.53
Food consumption (g/rat/day) Week 0-10	14.9 \pm 0.36	14.8 \pm 0.36	15.3 \pm 0.46	15.3 \pm 0.31

a Data were obtained from Tables IA-006, IA-011, IA-012, and IA-015 on pages 184, 189-190, and 193 of the study report.

- ii. **Gestation:** There were no effects of treatment on body weights, body weight gains, or food

consumption during gestation (Table 4c). Even though body weight gain was considered statistically significant for the 6.7 and 68.1 mg/kg/day groups, this finding was not considered adverse due to no dose-response and there was no corresponding effect on absolute body weight at those doses.

TABLE 4c. Mean (\pm SD) maternal body weights, body weight gains, and food consumption during gestation (F0 parental) ^a					
Observation/study day		Dose Group (mg/kg/day)			
		0	6.7	22.8	68.1
Body weight (g)	GD 0	219.8 \pm 14.44	218.6 \pm 14.66	220.8 \pm 13.84	221.5 \pm 13.68
	GD 7	243.5 \pm 15.53	242.0 \pm 17.17	241.8 \pm 14.77	246.1 \pm 15.57
	GD 14	267.9 \pm 17.59	268.3 \pm 18.80	266.7 \pm 15.93	272.4 \pm 18.07
	GD 20	322.9 \pm 24.21	330.9 \pm 22.19	326.5 \pm 19.40	335.1 \pm 24.74
Body weight gain (g)	GD 0-21	103.0 \pm 14.99	112.3 \pm 11.44* (\downarrow 9%)	105.6 \pm 13.48	113.7 \pm 17.21* (\downarrow 9.4%)
Food consumption (g/rat/day)	GD 0-21	19.4 \pm 1.37	19.7 \pm 1.75	19.7 \pm 1.85	20.4 \pm 1.65

a Data were obtained from Tables IA-007, IA-016, and IA-017 on pages 185 and 194-195 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.

* $p \leq 0.05$

iii. **Lactation:** There were no effects of treatment on body weights, body weight gains, or food consumption during lactation (Table 4d).

TABLE 4d. Mean (\pm SD) maternal body weights, body weight gains, and food consumption during lactation (F0 parental) ^a					
Observation/study day		Dose Group (mg/kg/day)			
		0	6.7	22.8	68.1
Body weight (g)	LD 0	240.8 \pm 14.86	243.6 \pm 20.36	245.0 \pm 18.48	245.6 \pm 17.65
	LD 4	255.0 \pm 14.70	258.1 \pm 20.08	257.0 \pm 16.70	259.8 \pm 17.86
	LD 7	257.9 \pm 15.31	259.1 \pm 12.79	261.6 \pm 17.43	262.9 \pm 13.00
	LD 14	277.1 \pm 15.54	280.3 \pm 16.46	281.2 \pm 18.79	285.6 \pm 13.80
	LD 21	255.9 \pm 18.72	259.1 \pm 15.95	260.4 \pm 22.13	269.8 \pm 16.96* (\uparrow 5.2%)
Body weight gain (g)	LD 0-21	15.1 \pm 20.58	15.5 \pm 24.76	15.4 \pm 18.05	23.4 \pm 17.83
Food consumption (g/rat/day)	LD 0-21	45.5 \pm 13.47	48.0 \pm 14.11	46.0 \pm 13.96	48.2 \pm 14.46

a Data were obtained from Tables IA-008, IA-018, IA-019 on pages 186, and 196-197 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.

* $p \leq 0.05$

3. **Reproductive function**

a. **Estrous cycle length and periodicity:** There were no effects of treatment on estrous cycle length or periodicity.

b. **Sperm measures:** There were no effects of treatment on sperm motility or sperm density (Table 5).

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Table 5: Sperm parameters of F ₀ parental males administered BAS 850 H on week 16 ^a						
Generation	F ₀ parental males				HCD [#]	
Dose [mg/kg/day]	0	6.4	21.5	64.4	[mean]	[range]
Animals per dose	30	30	29	30		
Sperm count [10 ⁶ / g]						
- Testis	141±33	138±28	133±40	131±27	-	-
- Cauda epididymis	738±130	673±135	687±185	728±150	-	-
Abnormal sperm [%]	6.6±1.0	6.5±1.1	6.6±1.0	6.8±1.4	6.2	6.0 – 7.5
Sperm motility [%]	87±5	87±6	87±7	87±5	-	-

^a Data were obtained from Table IB 17 on page 352 of the study report.

* = p<0.05; * = p<0.01; Wilcoxon with Bonferroni-Holm (one-sided, +/-)

[#] Historical Control Data based on 26 generational reproductive toxicity studies on Wistar Rats that were performed in the Laboratory of BASF SE between 2009 and 2014.

4. **Reproductive performance:** There were no effects of treatment on reproductive performance (Table 6).

Table 6: Reproduction parameters of F ₀ and F ₁ (=Cohort 1B) parental rats treated with BAS 850 H ^a								
Parental generation	F ₀				F ₁			
Dose (mg/kg/day)	0	6.7	22.8	68.1	0	6.7	22.8	68.1
Animals per dose	30	30	30	30	25	25	25	25
Male fertility								
- placed with females	29 ^b	30	30	30	25	25	25	25
- mated [n]	29	30	30	30	25	25	24	25
- Mating index [%]	100	100	100	100	100	100	96	100
- with females pregnant [n]	28	30	25	27	25	24	23	25
- Fertility index [%]	97	100	83	90	100	96	92	100
Female fertility								
- placed with males	30	30	30	30	25	25	25	25
- mated [n]	30	30	30	30	25	25	24	25
- Mating index [%]	100	100	100	100	100	100	96	100
- pregnant [n]	29	30	25	27	25	24	23	25
- Fertility index [%]	97	100	83	90	100	96	96	100
- Duration of gestation (days)	22.2±0.38	22.1±0.31	22.3±0.54	22.2±0.40	22.1±0.33	22.2±0.41	22.0±0.21	22.2±0.41
- Post implantation loss (total)	18±0.9	22±0.78	19±1.16	18±0.83	18±0.98	15±1.01	14±0.72	21±1.14
- Post implantation loss (%)	5.4±8.29	5.6±6.03	8.0±17.07	4.8±6.12	5.6±7.64	4.6±7.86	5.0±5.98	7.1±9.68
HCD [#] [range]	Male and female fertility index: 90 – 100%							

* = p<0.05; * = p<0.01; Fisher's exact test (two-sided);

[#] Historical control data based on 25 generational reproductive toxicity studies on Wistar rats supplied by Charles River that were performed in the Laboratory of BASF SE between 2007 and 2012.

^a Data were obtained from Tables IA-027, IA-028, IA-088, and IA-089 on pages 205, 206, 266, 267 of the study report, respectively.

^b There is an apparent discrepancy between the number of males/females placed with females/males and for females pregnant in the upper and lower part of the table. This is due to the fact that for reasons not specified in the report control male #3 was not mated to control female #203. Instead of male #3, male #6 was mated first mated with female #203 and, after successful mating within one day, was paired to female #206 (see Table IIA-125, report page 584).

5. **Clinical pathology:** There were no effects of treatment on hematology, clinical chemistry, urinalysis, or thyroid hormone concentrations (Table 7). Investigations of F₀ parental animals revealed a number of statistically significant differences; however, these effects were not considered adverse as they were only slightly decreased (<5%); within the historical controls; and/or lacked a dose response. The dose dependent decrease of serum bilirubin levels in mid and high dose males and females was likely due to the increased conjugation rate as consequence of the liver enzyme induction and accelerated excretion of bilirubin via the bile. This was regarded as an adaptive rather than an adverse effect. GGT increased in the high dose; however, these values were within the historical control range and were not considered adverse. Urea was

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increased in high dose females; however, this was not considered adverse due to no other corroborating effects. There was no effect on T4 levels or TSH levels of F0 males, as there was no dose response or statistical significance. However, T4 levels decreased by 27.5% in the high-dose females, which is considered adverse with the corroborating histopathological effects.

Table 7: Selected clinical chemistry findings of F ₀ parental rats on day 110 or 131 of BAS 850 H treatment ^a											
Generation / Sex	F ₀ Males				F ₀ Females				HCD [#]		
Dose [ppm]	0	6.4	21.5	64.4	0	6.7	22.7	68.1			
Animals per dose	10	10	10	10	10	10	10	10	sex	[mean]	[range]
	Day 110				Day 131				~ Day 126		
ALT [μkat/L]											
mean	0.7	0.7	0.7	0.8	0.52	0.59*	0.59*	0.62**	M	-	-
SD	0.14	0.15	0.11	0.26	0.05	0.08	0.06	0.09	F	0.58	0.48 - 0.72
Δ%		1.4	1.4	15.9		13.5	13.5	19.2			
ALP [μkat/L]											
mean	1.2	1.33	1.27	1.07	0.75	0.77	0.69	0.59	M	-	-
SD	0.13	0.22	0.26	0.11	0.25	0.19	0.16	0.14	F	-	-
Δ%		10.8	5.8	-10.8		2.7	-8	-21.3			
GGT [μkat/L]											
mean	0	0	0	6**	0	0	0	9**	M	1	0 - 6
SD	0	0	0	6	0	0	0	10	F	2	0 - 10
Urea [mmol/L]											
mean	6.97	6.63	6.78	6.78	6.58	6.96	7.14	8.07**	M	-	-
SD	0.91	0.86	1.18	1.22	0.88	1.55	1.1	0.77	F	6.52	5.31 - 7.80
Δ%		-4.9	-2.7	-2.7		5.8	8.5	22.6			
Bilirubin [μmol/L]											
mean	1.75	1.49	1.16**	0.95*	1.77	1.65	1.41*	1.10**	M	-	-
SD	0.38	0.32	0.16	0.13	0.33	0.31	0.3	0.21	F	-	-
Δ%		-14.9	-33.7	-45.7		-6.8	-20.3	-37.9			
Protein, total [g/L]											
mean	62.5 ₃	63.66	65.34*	63.77	63.7 ₃	62.6 ₉	65.49	68.78**	M	-	-
SD	2.11	1.47	1.88	2.15	1.5	2.06	2.53	2.86	F	65.97	62.13 - 70.12
Δ%		1.8	4.5	2		-1.6	2.8	7.9			

Table 7: Selected clinical chemistry findings of F₀ parental rats on day 110 or 131 of BAS 850 H treatment^a

Generation / Sex	F ₀ Males				F ₀ Females				HCD [#]		
Dose [ppm]	0	6.4	21.5	64.4	0	6.7	22.7	68.1			
Animals per dose	10	10	10	10	10	10	10	10	sex	[mean]	[range]
Albumin [g/L]											
mean	38.1 ₂	39.24 [*]	39.76 [*]	39.04 [*]	39.5 ₆	39.0 ₂	40.55	41.99 ^{**}	M	38.4	34.62 - 41.09
SD	1	1.02	0.73	0.82	0.76	1.2	1.74	1.66	F	41.41	37.96 - 43.65
Δ%		2.9	4.3	2.4		-1.4	2.5	6.1			
Globulin [g/L]											
mean	24.4 ₁	24.42	25.58	24.73	24.1 ₇	23.6 ₇	24.94	26.79 ^{**}	M	-	-
SD	1.42	0.84	1.34	1.55	1.06	1.14	1.27	1.45	F	24.72	19.54 - 29.31
Δ%		0.04	4.8	1.3		-2.1	3.2	10.8			
Chol [mmol/L]											
mean	1.96	2.03	1.87	1.74	1.5	1.52	1.66	1.82	M	-	-
SD	0.56	0.46	0.32	0.35	0.28	0.3	0.41	0.21	F	-	-
Δ%		3.6	-4.6	-11.2		1.3	10.7	21.3			
Trig [mmol/L]											
mean	0.76	1.01 [*]	0.86	0.74	0.82	1.01	0.91	1.16	M	-	-
SD	0.09	0.28	0.15	0.21	0.12	0.42	0.28	0.32	F	-	-
Δ%		32.9	13.2	-2.6		23.2	11	41.5			
INP [mmol/L]											
mean	1.55	1.45	1.48	1.51	1.03	1.21	1.21 [*]	1.36 ^{**}	M	-	-
SD	0.15	0.16	0.18	0.14	0.19	0.12	0.12	0.12	F	1.32	1.14 - 1.55
Δ%		-6.5	-4.5	-2.6		17.5	17.5	32			
CA [mmol/L]											
mean	2.47	2.53	2.53	2.5	2.49	2.5	2.53	2.58 ^{**}	M	-	-
SD	0.04	0.06	0.07	0.06	0.05	0.03	0.05	0.06	F	2.58	2.46 - 2.72
Δ%		2.4	2.4	1.2		0.4	1.6	3.6			
T ₄ [nmol/L]											
mean	85.2 ₆	85.76	82.26	79.92	63.0 ₁	52.9 ₁	53.75	45.71	M	-	-
SD	13.7 ₃	14.66	10.85	8.11	16.5 ₆	7.5	13.15	10.74	F	-	-
Δ%		0.6	-3.5	-6.3		-16	-14.7	-27.5			

Table 7: Selected clinical chemistry findings of F ₀ parental rats on day 110 or 131 of BAS 850 H treatment ^a										
Generation / Sex	F ₀ Males				F ₀ Females				HCD [#]	
Dose [ppm]	0	6.4	21.5	64.4	0	6.7	22.7	68.1		
Animals per dose	10	10	10	10	10	10	10	10	sex	[mean] [range]
TSH [μ g/L]										
mean	7.81	8.26	9.61	8.18	5.58	5.39	5.31	6.01	M	- -
SD	2.12	2.66	4.46	2.15	1.27	0.86	0.93	1.29	F	- -
$\Delta\%$		5.8	23	4.7		-3.4	-4.8	7.7		

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis + Wilcoxon test, two sided)

[#] Historical Control Data based on repeated dose toxicity studies on male and female Wistar rats (age of 18 weeks) that were performed in the Laboratory of BASF SE between 2009 and 2014.

^a Data obtained from Tables IB 5-IB 12 on pages 340-347 of the study report.

6. Parental postmortem results

- a. **Organ weights:** Selected organ weights are presented in Table 8. Treatment-related changes of absolute and/or relative organ weights were restricted to the liver in the mid- and high-dose F₀ parental males and females and to the thyroid of high dose males. The weights of these organs exceeded the historical control range. However, the changes in the liver were not accompanied by corroborative adverse enzyme effects, thus this finding is not considered adverse. Changes in the thyroid weights were accompanied by thyroid hyperplasia (see Table 9); however, the differences are not biologically significant because (1) percent change from control is within the variability of the controls, and (2) the high dose is only 1.3 mg absolute above historical control and within relative historical controls. Furthermore, the changes of absolute and/or relative adrenal gland, kidney and spleen weights in F₀ parental males and or females were either marginal (relative kidney weight high dose males), not dose dependent (absolute and relative spleen weights in treated males) or within the historical control range (adrenal glands). There were no corroborative histopathological findings in any case; therefore, these findings were not considered adverse.

Table 8: Selected organ weights of F ₀ parental animals ^a									
Generation / Sex		F ₀ Males				F ₀ Females			
Dose [mg/kg/day]		Absolute weight	$\Delta\%$ ^{&}	Relative weight [% of bw]	$\Delta\%$ ^{&}	Absolute weight	$\Delta\%$ ^{&}	Relative weight [% of bw]	$\Delta\%$ ^{&}
Terminal weight [g]	0	385 \pm 29				229 \pm 13			
	6.7	396 \pm 38	(+3.1)			227 \pm 14	(-0.8)		
	22.8	387 \pm 32	(+0.6)			228 \pm 15	(-0.4)		
	68.1	386 \pm 36	(+0.5)			229 \pm 15	(+0.3)		
Adrenal gland [mg]	0	57.9 \pm 6.8		0.015 \pm 0.002		72.7 \pm 8.7		0.03 \pm 0.004	
	6.7	58.6 \pm 7.6	(+1.2)	0.015 \pm 0.002	(\pm 0.0)	76.8 \pm 9.2	(+5.7)	0.03 \pm 0.004*	(+6.3)
	22.8	62.5 \pm 8.5*	(+7.9)	0.016 \pm 0.003	(+6.7)	77.2 \pm 10.9	(+6.2)	0.03 \pm 0.005	(+6.3)
	68.1	62.5 \pm 7.3*	(+7.9)	0.016 \pm 0.002*	(+6.7)	79.9 \pm 7.3**	(+10.0)	0.04 \pm 0.003**	(+9.4)
HCD [#]		55.920 – 71.400		0.014 – 0.020		72.100 – 85.200		0.031 – 0.037	
Kidneys [g]	0	2.5 \pm 0.2		0.64 \pm 0.04		1.7 \pm 0.14		0.73 \pm 0.04	
	6.7	2.5 \pm 0.2	(+0.8)	0.63 \pm 0.04	(-1.9)	1.7 \pm 0.12	(-0.7)	0.73 \pm 0.04	(+0.3)
	22.8	2.5 \pm 0.2	(+1.9)	0.65 \pm 0.04	(+1.3)	1.7 \pm 0.15	(+1.0)	0.74 \pm 0.06	(+1.6)
	68.1	2.6 \pm 0.3	(+4.1)	0.66 \pm 0.06*	(+3.9)	1.7 \pm 0.11	(+2.8)	0.75 \pm 0.04	(+2.6)

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Table 8: Selected organ weights of F ₀ parental animals ^a									
Generation / Sex		F ₀ Males				F ₀ Females			
	Dose [mg/kg/ day]	Absolute weight Δ%&		Relative weight [% of bw] Δ%&		Absolute weight Δ%&		Relative weight [% of bw] Δ%&	
HCD [#]		2.192 – 2.631		0.569 - 0.696					
Liver	[g] 0	8.7±1.0		2.3±0.15		6.0±0.5		2.6±0.2	
	6.7	9.1±1.1	(+4.5)	2.3±0.11	(+1.4)	6.1±0.5	(+1.9)	2.7±0.1	(+2.6)
	22.8	9.6±1.1**	(+10.2)	2.5±0.12**	(+9.3)	6.5±0.6**	(+8.1)	2.8±0.2**	(+8.7)
	68.1	10.8±1.3**	(+23.5)	2.8±0.15**	(+23.0)	7.6±0.7**	(+27.3)	3.3±0.3**	(+26.9)
HCD [#]		8.144 – 9.327		2.149 – 2.507		5.709 – 7.649		2.512 – 3.179	
Spleen	[g] 0	0.6±0.1		0.15±0.02		0.48±0.06		0.21±0.02	
	6.7	0.6±0.1*	(+8.2)	0.16±0.02*	(+4.7)	0.46±0.08	(-4.0)	0.20±0.03	(-3.3)
	22.8	0.6±0.09	(+5.6)	0.16±0.02*	(+4.7)	0.47±0.06	(-0.8)	0.21±0.02	(-0.5)
	68.1	0.7±0.1**	(+13.9)	0.17±0.02**	(+13.4)	0.47±0.06	(-0.8)	0.21±0.02	(-1.4)
HCD [#]		0.556 – 0.654		0.144 – 0.173					
Thyroids	[mg] 0	22.5 ± 5.4		0.006 ± 0.001		16.9 ± 3.4		0.007 ± 0.001	
	6.7	26.8 ± 9.1	(+19)	0.007 ± 0.002	(+17)	16.9 ± 2.3	(+0)	0.007 ± 0.001	(±0)
	22.8	25.0 ± 4.8	(+11)	0.006 ± 0.001	(± 0)	18.2 ± 3.1	(+8)	0.008 ± 0.001	(+14)
	68.1	28.0 ± 6.0**	(+24)	0.007 ± 0.001**	(+17)	18.8 ± 3.7	(+11)	0.008 ± 0.002	(+14)
HCD [#]		19.0 – 26.7		0.005 – 0.008		-		-	

* p ≤ 0.05. ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test (two-sided))

& Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means

^a Data were obtained from Tables IC 1/51 – IC 8/51 on pages 381-388 of the study report.

[#] Historical Control Data (range values) based on 14 to 21 generational reproductive toxicity studies on male and female Wistar rats (F₀ generation) that were performed in the Laboratory of BASF SE between 2012 and 2015.

b. Pathology

- i. **Macroscopic examination:** There were no treatment-related gross pathological findings.
- ii. **Microscopic examination:** Selected microscopic findings are presented in Table 9. There is an increase in incidence of liver effects at the high dose for both males and females. However, these effects were not coupled with adverse enzyme effects and are not considered toxicologically adverse. Hypertrophy/hyperplasia and altered colloid was observed in the thyroid gland for all dose groups. These effects are considered adverse in the mid- and high-dose males and females due to an increase in severity and incidence.

Table 9: Selected histopathological findings of relevant organs of F ₀ parental rats administered BAS 850 H ^a									
Dose level (mg/kg/day)		F ₀ Males				F ₀ Females			
		0	6.4	21.5	64.4	0	6.7	22.8	68.1
Animals examined		30	30	30	30	30	30	30	30
Liver									
- Multinucleated hepatocytes					15		1	4	6
Minimal					7		1	3	6
Slight					7			1	
Moderate					1				
Average					[1.6]		[1.0]	[1.3]	[1.0]
- Necrosis, single cell					2				
Minimal					1				
Slight					1				
Moderate									
Average					[1.5]				

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Table 9: Selected histopathological findings of relevant organs of F ₀ parental rats administered BAS 850 H ^a								
Dose level (mg/kg/day)	F ₀ Males				F ₀ Females			
	0	6.4	21.5	64.4	0	6.7	22.8	68.1
- Hypertrophy, centrilobular								5
Minimal								5
Slight								
Moderate								
Average								[1.0]
- Hypertrophy, diffuse				13				10
Minimal				11				9
Slight				2				1
Moderate								
Average				[1.2]				[1.1]
- Pigment, hepatocytes	1			14				13
Minimal	1			14				12
Slight								1
Moderate								
Average	[1.0]			[1.0]				[1.1]
Thyroid glands								
- Hypertrophy/hyperplasia, follicular (epithelial cells)	4	3	10	17	1	2	1	20
Minimal	4	3	7	7	1	2	1	11
Slight			2	8				8
Moderate			1	2				1
Average	[1.0]	[1.0]	[1.4]	[1.7]	[1.0]	[1.0]	[1.0]	[1.5]
- Altered colloid	17	17	22	30	21	16	13	30
Minimal	13	16	11	7	17	16	12	8
Slight	4	1	6	9	4		1	15
Moderate			5	14				6
Marked								1
Average	[1.2]	[1.1]	[1.7]	[2.2]	[1.2]	[1.1]	[1.1]	[2.0]

^a Data obtained from p. 153-154 and 395 of study report.

= mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding

B. F1 GENERATION PUPS

- Viability and clinical signs:** Litter parameters are presented in Table 10. There were no adverse effects on litter parameters.

Table 10: Litter parameters ^a								
Pup generation	F ₁				F ₂			
	0	6.7	22.8	68.1	0	6.7	22.8	68.1
Number of litters	29	30	25	27	25	24	23	25
- with liveborn pups	29 ^b	30	24	27	25	24	23	25
- with stillborn pups	2	3	4	0	2	2	1	3
Pups liveborn [n]	323 (11)	364 (12)	269 (11)	332 (12)	277 (11)	288 (12)	269 (12)	280 (11)
Pups stillborn [n]	2	3	5	0	3	2	1	4
Pups died [n]	0	3	0	5*	0	4	0	2
[%]	0.0	0.8	0.0	1.5	0.0	1.4	0.0	0.7
Pups cannibalized [n]	1	2	1	6	3	3	2	3
[%]	0.3	0.5	0.4	1.8	1.1	1.0	0.7	1.1
HCD# [range]	perinatal loss [mean]: 0.0 – 5.6%							
Pups culled day 4 [n]	47	60	42	64	35	45	44	42
Pups day 4 - pre-cull [n]	322	359	268	324*	274	281	267	276
- Viability index [%]	100	99	100	98	99	98	99	99
HCD# [range]	Viability index [mean]: 94 – 100%							

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Table 10: Litter parameters ^a								
Pup generation	F ₁				F ₂			
Dose (mg/kg/day)	0	6.7	22.8	68.1	0	6.7	22.8	68.1
Pups day 4 - post cull [n]	275	299	226	260	239	236	223	234
Pups day 21 [n]	275	299	226	257	239	236	223	233
- Lactation index [%]	100	100	100	99	100	100	100	100
Sex ratio [% live males]								
- Day 0	51.4	51.9	53.9	50.3	44.0	52.1	49.8	44.3
- Day 21	49.5	51.5	53.5	49.8	46.0	53.4	49.8	46.4

* p ≤ 0.05; ** p ≤ 0.01 Dunnett-test, two sided

^a Data obtained from Tables IA-030-IA-032 on pages 208-210 from the study report.

^b Only 28 males were used because 1 male was accidentally mated twice

[#] Historical Control Data based on 25 Generational Reproductive Toxicity Studies on Wistar Rats supplied by Charles River that were performed in the Laboratory of BASF SE between 2007 and 2012.

Data in parenthesis is based on a per litter basis

2. **Developmental landmarks:** There were no effects of treatment observed on the presence of nipples in male pups on PND 20; no males in any group had nipples at this age. Anogenital distances measured on PND 0 and 21 in both male and female pups were unaffected by treatment (means: 3.01-3.17 mm males, 1.39-1.57 mm females). Mean age (PND) at balanopreputial separation was about 42 days (SD 1.45-1.96) for all groups (Table 11). Mean age at vaginal patency was about 30-31 days (SD 1.17-1.42) for all groups (Table 11).

Table 11: Sexual maturation of F1 pups (F1 cohort 1A, 1B, 2A and 3) ^a								
Sex & Parameter	Females / Vaginal opening				Males / Preputial separation			
Dose [mg/kg/day]	0	6.7	22.8	68.1	0	6.4	21.5	64.4
Animals per dose	65	65	65	65	65	65	65	65
- Days to criterion (mean)	30.5	31.0	31.0	31.1*	42.0	42.3	42.4	42.4
(SD)	1.17	1.32	1.42	1.27	1.79	1.65	1.96	1.45
HCD [#] (range)	females: 29.5 – 31.9 days				males: 40.5 – 45.2 days			
- Body weight at criterion (mean)	84.7	89.3*	91.3**	89.2*	167.3	174.4*	173.7	172.7
(SD)	4.77	4.36	7.41	5.46	7.58	9.31	14.62	7.79
HCD [#] (range)	females: 83.1 – 100.7 g				males: 168.1 – 195.3 g			

* p ≤ 0.05; ** p ≤ 0.01 (Dunnett-test, two-sided)

[#] Historical Control Data based on 17 Generational Reproductive Toxicity Studies on Wistar Rats supplied by Charles River that were performed in the Laboratory of BASF SE between 2010 and 2015.

^a Data were obtained from Tables IA-045 and IA-047 on page 223 and 225, respectively from the study report.

3. **Body weight:** F1 and F2 pup body weights and body weight gains are presented in Table 12. There were no adverse effects on body weight and body weight gains.

Table 12: Mean body weights (g) ^a								
Pup generation	F ₁				F ₂			
Dose (mg/kg/day [M/F])	0/0	6.4/6.7	21.5/22.8	64.4/68.1	0/0	6.4/6.7	21.5/22.8	64.4/68.1
Number of litters	29	30	25	27	25	24	23	25
Male pup weight [g]								
- day 1 [g]	6.7±0.75	6.7±0.46	6.8±0.77	6.6±0.58	6.7±0.64	6.9±0.51	6.6±0.60	6.9±0.78
- day 4 – pre-cull [g]	10.1±1.38	10.2±0.74	10.7±1.32	10.2±1.03	10.0±1.14	10.3±1.08	9.9±1.11	10.4±1.36
- day 4 – post-cull [g]	10.2±1.37	10.2±0.73	10.7±1.32	10.2±0.99	10.0±1.13	10.4±1.06	9.9±1.11	10.4±1.36
- day 7 [g]	15.3±1.94	15.5±1.01	16.0±1.60	15.5±1.19	14.8±1.59	15.5±1.27	15.1±1.47	15.8±1.48*
- day 14 [g]	29.4±2.92	30.2±1.82	30.7±2.42	30.2±2.18	28.5±2.85	30.1±2.34	29.9±2.57	30.7±2.53*
- day 21 [g]	45.8±4.14	46.9±2.58	48.8±3.61**	47.8±3.25	46.5±3.88	47.9±3.65	47.6±3.65	48.5±4.46
Male body weight gain								
- day 4 to 7 [g]	5.2±0.85	5.3±0.63	5.3±0.60	5.3±0.56	4.8±0.68	5.2±0.54	5.2±0.62	5.4±0.61**
[Δ%]		+1.9	+1.9	+1.9		+8.3	+8.3	+12.5

Table 12: Mean body weights (g) ^a								
Pup generation	F ₁				F ₂			
Dose (mg/kg/day [M/F])	0/0	6.4/6.7	21.5/22.8	64.4/68.1	0/0	6.4/6.7	21.5/22.8	64.4/68.1
- day 7 to 14 [g]	14.1±1.34	14.7±1.19	14.7±1.19	14.7±1.31	13.7±1.65	14.6±1.56	14.9±1.49*	14.9±1.46*
[Δ%]		+4.3	+4.3	+4.3		+6.6	+8.8	+8.8
- day 14 to 21 [g]	16.4±2.26	16.7±1.66	18.2±1.67**	17.6±1.49*	18.0±1.47	17.8±1.88	17.7±1.45	17.7±2.60
[Δ%]		+1.8	+11.0	+7.3		-1.1	-1.7	-1.7
- day 4 to 21 [g]	35.7±3.08	36.7±2.26	38.1±2.56**	37.6±2.84*	36.5±3.05	37.6±3.08	37.8±2.92	38.1±3.75
[Δ%]		+2.8	+6.7	+5.3		+3.0	+3.6	+4.4
Female pup weight [g]								
- day 1 [g]	6.5±1.09	6.4±0.41	6.6±0.82	6.4±0.57	6.4±0.61	6.5±0.46	6.3±0.56	6.5±0.80
- day 4 – pre-cull [g]	9.8±1.43	9.9±0.72	10.4±1.43	9.9±1.06	9.6±1.14	9.9±0.96	9.6±1.07	10.0±1.34
- day 4 – post-cull [g]	9.9±1.40	9.9±0.72	10.4±1.39	10.0±1.04	9.7±1.13	9.9±0.96	9.6±1.09	10.0±1.28
- day 7 [g]	14.9±1.94	15.0±0.93	15.5±1.67	15.1±1.24	14.4±1.58	15.0±1.13	14.6±1.38	15.2±1.54
- day 14 [g]	28.7±2.63	29.5±1.64	29.9±2.58	29.5±2.18	28.0±2.66	29.4±2.22	29.2±2.46	29.8±2.65*
- day 21 [g]	44.7±3.81	46.1±2.42	47.5±3.70**	46.7±3.26	45.4±3.77	46.7±3.12	46.6±3.56	47.0±4.19
Female body weight gain								
- day 4 to 7 [g]	5.1±0.83	5.2±0.65	5.1±0.57	5.1±0.59	4.7±0.65	5.1±0.55	5.0±0.57	5.2±0.59**
[Δ%]		+2.0	±0.0	±0.0		+8.5	+6.4	+10.6
- day 7 to 14 [g]	13.8±1.16	14.5±1.09*	14.4±1.21	14.4±1.31	13.6±1.56	14.4±1.66	14.6±1.59	14.6±1.52
[Δ%]		+5.1	+4.3	+4.3		+5.9	+7.4	+7.4
- day 14 to 21 [g]	16.0±2.26	16.6±1.49	17.6±1.61**	17.2±1.57*	17.4±1.53	17.3±1.35	17.4±1.50	17.2±2.27
[Δ%]		+3.8	+10.0	+7.5		-0.6	±0.0	-1.1
- day 4 to 21 [g]	34.9±2.74	36.3±2.20	37.1±2.49**	36.8±2.84*	35.8±2.90	36.8±2.77	37.0±3.04	37.0±3.40
[Δ%]		+4.0	+6.3	+5.4		+2.8	+3.4	+3.4

* p ≤ 0.05; ** p ≤ 0.01 Dunnett-test, two sided

^a Data obtained from Tables IA-035 – IA-038 on pages 213-216 for the F₁ data and Tables IA-096-IA-099 on pages 274-277 for the F₂ data of the study report

4. **Clinical pathology:** There was no effect on T4 or TSH observed in F₁ pups at PND4 (culled) or in PND22 (surplus animals) (Table 13). Review of the calibration curves indicate these measurements were within the sensitivity of the assays.

Table 13: Thyroid hormones findings of F ₁ pups on PND 4 and PND 22 ^a								
Pup Generation / Sex	F ₁ male pups				F ₁ female pups			
Dose [mg/kg/day]	0	6.4	21.5	64.4	0	6.7	22.8	68.1
Animals per dose	10	10	10	10	10	10	10	10
PND 4								
T ₄ [nmol/L]								
mean	41.48	46.34	41.87	45.15	44.11	39.71	44.40	47.13
SD	9.74	8.74	10.16	9.82	12.28	11.19	7.89	7.33
Δ%		+11.7	+0.9	+8.8		-10.0	+0.7	+6.8
TSH [μg/L]								
mean	7.25	7.57	7.56	7.92	7.58	7.92	7.85	7.79
SD	1.17	1.01	0.82	0.68	0.93	0.71	0.72	1.02
Δ%		+4.4	+4.3	+9.2		+4.5	+3.6	+2.8
PND 22								
T ₄ [nmol/L]								
mean	89.03	81.89	84.00	82.28	87.25	82.11	83.36	82.87
SD	14.12	13.76	13.96	16.42	13.10	10.55	15.36	10.81
Δ%		-8.0	-5.6	-7.6		-5.9	-4.5	-5.0
TSH [μg/L]								
mean	5.00	4.50	4.80	5.35	4.51	4.45	5.17	5.02
SD	0.64	0.82	1.15	0.99	0.64	0.87	0.92	0.84
Δ%		-10.0	-4.0	+7.0		-1.3	+14.6	+11.3

^a Data obtained from Table IB 18- IB 21 on pages 353-356 from the study report.

5. Post-mortem results

- a. **Organ weights:** There were no effects of treatment on absolute and relative pup organ weights (brain, thymus, and spleen) in the F2 pups.
- b. **Pathology**
- i. **Macroscopic examinations:** Macroscopic findings in Cohort 1A animals were rare and consisted of renal pelvis dilation in one high dose male and female, torsion of the liver in one high dose female and reduced seminal vesicle size in one low dose male (Table 14). The single occurrence of these observations were assessed as incidental and unrelated to treatment. There were a number of gross necropsy findings in Cohort 1B pups. Most of the findings were either observed singly and/or without a dose response relationship or at an incidence comparable to or lower as in the concurrent or the historical control; therefore, these findings were not considered to be related to treatment. However, the incidence of dilated renal pelvises was significantly increased at the high dose level and was observed outside the historical control range. Dilated renal pelvises are common finding in untreated rats of the Wistar strain. In the affected pups no compression of the parenchyma surrounding the renal pelvis was observed, which would have been an indication for an obstructed urogenital tract. Thus, the most likely underlying mechanism of renal pelvis dilation is the transient inhibition of renal growth, particularly regarding the length of the renal papilla during late gestation. Additionally, literature references consistently report this kind of renal pelvis dilation to be reversible postnatally (Woo and Hoar, 1972). Therefore, this finding is considered of no toxicological relevance and was not considered as an adverse finding by itself.

Table 14: Incidence of selected gross necropsy observations in F2 pups ^a					
Dose [mg/kg/day]	0	6.4/6.7	21.5/22.8	64.4/68.1	HCD# [range]
	F2 pups				F1/F2 pups
Litters evaluated	25	24	23	25	589
Pups evaluated	275	287	264	281	6390
- Live	272	285	263	277	6329
- Stillborn	3	2	1	4	61
Post mortem autolysis					
- Pup incidence [No. (%)]	2 (0.7)	1 (0.3)	0 (0.0)	1 (0.4)	13 ^a (0.0 – 2.7) ^b
- Litter incidence [No. (%)]	2 (8.0)	1 (4.2)	0 (0.0)	1 (4.0)	6 ^a (0.0 – 8.3) ^b
- Affected fetuses/litter [Mean %]	0.7	0.3	0.0	0.3	0.2 ^c (0.0 – 2.7) ^b
Hemorrhagic thymus					
- Pup incidence [No. (%)]	0 (0.0)	0 (0.0)	3 (1.1)	0 (0.0)	4 ^a (0.0 – 0.6) ^b
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	2 (8.7)	0 (0.0)	4 ^a (0.0 – 8.0) ^b
- Affected fetuses/litter [Mean %]	0.0	0.0	1.2	0.0	0.1 ^c (0.0 – 0.6) ^b
Diaphragmatic hernia					
- Pup incidence [No. (%)]	1 (0.4)	1 (0.3)	3 (1.1)	1 (0.4)	3 ^a (0.0 – 0.5) ^b
- Litter incidence [No. (%)]	1 (4.0)	1 (4.2)	2 (8.7)	1 (4.0)	3 ^a (0.0 – 4.2) ^b
- Affected fetuses/litter [Mean %]	0.3	0.3	1.1	0.4	0.1 ^c (0.0 – 2.1) ^b
Dilated renal pelvis					
- Pup incidence [No. (%)]	2 (0.7)	2 (0.7)	3 (1.1)	16 (5.7)	26 ^a (0.0 – 2.3) ^b
- Litter incidence [No. (%)]	1 (4.0)	2 (8.3)	3 (13)	10 ^{**} (40)	20 ^a (0.0 – 20.0) ^b
- Affected fetuses/litter [Mean %]	0.9	0.7	1.2	6.1 ^{**}	0.4 ^c (0.0 – 2.1) ^b
Hydronephrosis					
- Pup incidence [No. (%)]	2 (0.7)	1 (0.3)	0 (0.0)	1 (0.4)	Not in HCD
- Litter incidence [No. (%)]	1 (4.0)	1 (4.2)	0 (0.0)	1 (4.0)	
- Affected fetuses/litter [Mean %]	0.9	0.3	0.0	0.3	

Table 14: Incidence of selected gross necropsy observations in F ₂ pups ^a					
Dose [mg/kg/day]	0	6.4/6.7	21.5/22.8	64.4/68.1	HCD [#] [range]
	F ₂ pups				F ₁ /F ₂ pups
Hydroureter					
- Pup incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	1 ^b (0.0 – 0.3) ^c
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)	1 ^b (0.0 – 4.0) ^c
- Affected fetuses/litter [Mean %]	0.0	0.0	0.0	0.3	0.0 ^d (0.0 – 0.4) ^c
Small testis					
- Pup incidence [No. (%)]	0 (0.0)	0 (0.0)	1 (0.4)	1 (0.4)	9 ^b (0.0 – 0.8) ^c
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	1 (4.3)	1 (4.0)	8 ^b (0.0 – 8.7) ^c
- Affected fetuses/litter [Mean %]	0.0	0.0	0.5	0.5	0.1 ^d (0.0 – 0.8) ^c
Small epididymis					
- Pup incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	Not in HCD
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)	
- Affected fetuses/litter [Mean %]	0.0	0.0	0.0	0.5	
Head, multiple malformations					
- Pup incidence [No. (%)]	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	Not in HCD
- Litter incidence [No. (%)]	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)	
- Affected fetuses/litter [Mean %]	0.0	0.3	0.0	0.0	
Total pup necropsy observations					
- Pup incidence [No. (%)]	7 (2.5)	6 (2.1)	10 (3.8)	20 (7.1)	79 ^b (0.0 – 3.7) ^c
- Litter incidence [No. (%)]	4 (16)	6 (25)	8 (35)	11 ^{**} (44)	64 ^b (0.0 – 28.0) ^c
- Affected fetuses/litter [Mean %]	2.8	1.9	4.1	7.6 ^{**}	1.3 ^d (0.0 – 3.8) ^c

() values in brackets give litter incidence

* = p<0.05; * = p<0.01; Fisher's exact test or Wilcoxon test (one-sided)

^a Data were obtained from Table IA-043 on page 221 of the study report.

[#] Historical Control Data based on 17 generational reproductive toxicity studies on Wistar Rats supplied by Charles River that were performed in the Laboratory of BASF SE between Jan 2007 and May 2011.

^b Number; ^c % range (min – max); ^d Mean %

ii. **Microscopic examinations:** Microscopic findings were not conducted for the F₁ pups.

C. **F1 ADULTS – COHORTS 1A AND 1B**

1. **Mortality and clinical signs**

a. **Mortality:** There were no test substance-related or spontaneous mortalities in any dose group for F1A or F1B.

b. **Clinical signs:** No clinical signs or changes of general behavior, which may be attributed to the test substance, were detected in any of the male and female animals in any of the groups. One low-dose male animal from F1A showed a spontaneous skin lesion (shoulder) in study week 6.

2. **Body weights, body weights gains, and food consumption:** Selected body weights, body weight gains, and food consumption are presented in Table 15. There were no adverse effects on body weights, body weight gains, or food consumption.

TABLE 15. Selected mean (\pm SD) body weights, body weight gains, and food consumption in the F1A generation^a

Observation/study day		Dose group (mg/kg/day)			
		0	6.7	22.8	68.1
Males					
Body weight (g)	Week 0	62.4 \pm 6.78	70.1 \pm 7.75**	65.5 \pm 7.30	64.9 \pm 7.66
	Week 2	150.9 \pm 9.16	161.5 \pm 13.0*	155.5 \pm 13.38	156.2 \pm 11.39
	Week 9	335.2 \pm 26.16	337.0 \pm 27.28	338.3 \pm 32.93	349.5 \pm 23.40
Body weight gain (g)	Week 0-9	272.8 \pm 24.49	267.0 \pm 23.66	272.8 \pm 29.25	284.7 \pm 21.76
Food consumption (g/rat/day)	Week 0-9	19.8 \pm 2.83	19.8 \pm 2.44	20.1 \pm 2.81	20.8 \pm 3.15
Females					
Body weight (g)	Week 0	60.2 \pm 7.66	65.3 \pm 6.08	62.4 \pm 6.36	61.1 \pm 7.07
	Week 2	129.2 \pm 12.38	131.9 \pm 9.59	130.8 \pm 9.38	124.1 \pm 8.45
	Week 9	213.8 \pm 16.01	211.2 \pm 14.85	206.1 \pm 11.47	203.3 \pm 10.56* (↓ 5%)
Body weight gain (g)	Week 0-9	153.5 \pm 13.2	145.9 \pm 13.56	143.7 \pm 8.79* (↓ 6%)	142.2 \pm 8.51** (↓ 7%)
Food consumption (g/rat/day)	Week 0-9	15.3 \pm 1.37	15.4 \pm 1.23	15.2 \pm 1.18	15.0 \pm 1.36

^a Data were obtained from Tables IA-050 - IA-055 on pages 228-233 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.

3. **Sexual maturation:** All pups selected to become a rearing F1 pup in Cohorts 1A, 1B, 2A and 3 were evaluated for commencement of the sexual maturation (Table 16). No effect on the mean number of days for reaching the sexual maturation criterion was noted in treated males and females. The mean number of days to reach vaginal opening was statistically significantly higher in high dose females. However, the mean value was within the historical control range and was not considered to be adverse. Likewise, the mean bodyweight at criterion was significantly increased in all treated female groups and in low dose males. However, these were considered to be incidental and not related to treatment due to a lack in dose-response and all values were well within the historical control range.

Table 16: Sexual maturation of F1 pups (F1 cohort 1A, 1B, 2A and 3) ^a									
Sex & Parameter		Females / Vaginal opening				Males / Preputial separation			
Dose	mg/kg/day	0	6.7	22.8	68.1	0	6.4	21.5	64.4
Animals per dose		65	65	65	65	65	65	65	65
- Days to criterion	(mean)	30.5	31.0	31.0	31.1*	42.0	42.3	42.4	42.4
	(SD)	1.17	1.32	1.42	1.27	1.79	1.65	1.96	1.45
	HCD [#] (range)	females: 29.5 – 31.9 days				males: 40.5 – 45.2 days			
- Body weight at criterion (mean)		84.7	89.3*	91.3**	89.2*	167.3	174.4*	173.7	172.7
	(SD)	4.77	4.36	7.41	5.46	7.58	9.31	14.62	7.79
	HCD [#] (range)	females: 83.1 – 100.7 g				males: 168.1 – 195.3 g			

* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett-test, two-sided)

^a Data obtained from Table IA-045 – IA-047 on pages 223-225 of the study report.

[#] Historical Control Data based on 17 Generational Reproductive Toxicity Studies on Wistar Rats supplied by Charles River that were performed in the Laboratory of BASF SE between 2010 and 2015.

4. Reproductive function

- a. **Estrous cycle length and periodicity:** There were no effects to cycle length and periodicity.
- b. **Sperm measures:** A slight and statistically significant increase ($p \leq 0.01$) of abnormal sperm above the historical control range was noted in high dose males (9% treated vs. 6.7% control) (Table 17) and was considered adverse.

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Table 17: Sperm parameters of Cohort 1A males administered BAS 850 H on Day 90 ^a						
Generation / Sex		Cohort 1A males				HCD [#]
Dose	[mg/kg/day]	0	6.4	21.5	64.4	[mean] [range]
Animals per dose		20	20	20	20	
Sperm count	[10 ⁶ / g]					
- Testis		99	109	106	109	
- Cauda epididymis		710	761	771	721	
Abnormal sperm	[%]	6.7	6.7	7.5	9.0**	6.2 6.0 – 7.5
- head bent	[%]	0.1	0.2	0.4	1.6**	
- tail missed	[%]	0.1	0.1	0.1	0.8**	
Sperm motility	[%]	88	87	88	85	

* = p<0.05; * = p<0.01; Wilcoxon with Bonferroni-Holm (one-sided, +/-)

[#] Historical Control Data based on 31 Generational Reproductive Toxicity Studies on Wistar Rats that were performed in the Laboratory of BASF SE between 2009 and 2014.

^a Data obtained from Table IB 42-IB 43 on pages 377-378 of the study report.

5. **Clinical chemistry:** In high dose Cohort 1A male and females, a number of treatment-related clinical chemistry changes were observed (statistically significant at p≤0.01) (Table 18). Even though increased total protein and globulin levels in both sexes as well as increased albumin, cholesterol and calcium levels in females were statistically significant, they were not considered toxicologically adverse because the values were either in the historical control range or the magnitude of change was minimal and not considered adverse. GGT was increased in high dose males as well as mid and high dose females. The effect at the high dose was considered adverse since it was not within the range of historical controls. Urea was significantly increased for the high dose females; however, this effect was in the historical control range and not considered toxicologically adverse. A slightly increased amount of blood was observed in the urine of the high-dose Cohort 1A males; however, there were no corroborating effects to indicate adversity. There was no effect on T4 or TSH at any dose tested (Table 19).

Table 18: Selected clinical chemistry findings of Cohort 1A (rearing) rats on day 90 of BAS 850 H treatment ^a										
Generation / Sex		Cohort 1A males				Cohort 1A females				HCD [#]
Dose	[mg/kg/day]	0	6.4	21.5	64.4	0	6.7	22.8	68.1	
Animals per dose		10	10	10	10	10	10	10	10	sex [mean] [range]
		Day 90				Day 90				~ Day 70
ALT [μkat/L]										
mean		0.77	0.77	0.77	0.76	0.73	0.66	0.70	0.68	M - -
SD		0.12	0.17	0.14	0.13	0.14	0.15	0.16	0.09	F 0.58 0.48 - 0.72
Δ%			±0.0	±0.0	-1.3		-9.6	-4.1	-6.8	
ALP [μkat/L]										
mean		1.68	1.72	1.46	1.43	0.89	0.95	0.73*	0.73	M - -
SD		0.36	0.36	0.19	0.32	0.23	0.21	0.16	0.10	F 1.26 0.71 - 1.80
Δ%			+2.4	-13.1	-14.9		+6.7	-18.0	-18.0	
GGT [μkat/L]										
mean		0	0	0	17**	0	0	6**	30**	M 1 0 - 6
SD		0	0	0	12	1	1	4	17	F 3 0 - 14
Urea [mmol/L]										
mean		5.63	5.30	5.75	6.54	5.61	5.81	5.75	6.86**	M - -
SD		0.90	0.92	0.71	1.12	0.73	1.13	0.58	1.07	F 6.52 5.29 - 7.84
Δ%			-5.9	+2.1	+16.2		+3.6	+2.5	+22.3	
Bilirubin [μmol/L]										
mean		1.87	1.48*	1.13**	0.90**	1.63	1.59	1.15**	0.95**	M - -
SD		0.36	0.30	0.20	0.15	0.21	0.30	0.22	0.09	F - -
Δ%			-20.9	-39.6	-51.9		-2.5	-29.4	-41.7	

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Table 18: Selected clinical chemistry findings of Cohort 1A (rearing) rats on day 90 of BAS 850 H treatment ^a												
Generation / Sex		Cohort 1A males				Cohort 1A females				HCD [#]		
Dose [mg/kg/day]	0	6.4	21.5	64.4	0	6.7	22.8	68.1				
Animals per dose	10	10	10	10	10	10	10	10	sex	[mean]	[range]	
	Day 90				Day 90				~ Day 70			
Protein, total [g/L]												
mean	64.07	65.75*	65.25	66.81**	62.47	64.07	65.27*	68.08**	M	61.03	58.22 – 64.31	
SD	1.28	1.62	1.69	1.67	1.86	1.12	2.29	1.39	F	61.63	58.40 – 65.33	
Δ%		+2.6	+1.8	+4.3		+2.6	+4.5	+9.0				
Albumin [g/L]												
mean	39.75	40.08	39.79	40.72	39.55	40.74	41.53**	42.34**	M	38.40	34.62 - 41.09	
SD	0.99	1.33	1.09	1.09	1.53	0.67	1.25	0.68	F	39.56	37.33 – 41.94	
Δ%		+0.8	+0.1	+2.4		+3.0	+5.0	+7.1				
Globulin [g/L]												
mean	24.32	25.66*	25.45	26.09**	22.92	23.32	23.75	25.74**	M	23.19	20.11 – 26.62	
SD	1.13	1.02	1.19	1.19	0.67	0.63	1.38	1.21	F	24.72	19.54 - 29.31	
Δ%		+5.5	+4.6	+7.3		+1.7	+3.6	+12.3				
Chol [mmol/L]												
mean	1.85	2.02	1.64	1.70	1.36	1.53	1.52	1.87**	M	-	-	
SD	0.40	0.28	0.27	0.26	0.32	0.44	0.25	0.32	F	-	-	
Δ%		+9.2	-11.4	-8.1		12.5	+11.8	+37.5				
Trig [mmol/L]												
mean	0.75	0.78	0.68	0.61	0.45	0.57	0.51	0.57	M	-	-	
SD	0.22	0.32	0.21	0.30	0.11	0.24	0.08	0.20	F	-	-	
Δ%		+4.0	-9.3	-18.7		26.7	+13.3	+26.7				
INP [mmol/L]												
mean	1.82	1.98*	1.84	1.97*	1.50	1.49	1.54	1.71*	M	2.16	1.83 – 2.39	
SD	0.11	0.15	0.16	0.16	0.21	0.17	0.22	0.11	F	1.71	1.41 – 2.08	
Δ%		+8.8	+1.1	+8.2		-0.7	+2.7	+14.0				
CA [mmol/L]												
mean	2.61	2.66	2.66	2.66	2.59	2.59	2.63	2.68**	M	-	-	
SD	0.06	0.06	0.07	0.07	0.04	0.06	0.09	0.04	F	2.58	2.46 - 2.72	
Δ%		+1.9	+1.9	+1.9		±0.0	+1.5	+3.5				

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis + Wilcoxon test, two sided)

[#] Historical Control Data based on repeated dose toxicity studies on male and female Wistar rats (age of 10 weeks) that were performed in the Laboratory of BASF SE between 2009 and 2014.

^a Data obtained from IB 30- IB 35 on pages 365-370 of the study report.

Table 19: Thyroid hormones findings of Cohort 1A (rearing) rats on day 90 of BAS 850 H treatment ^a									
Generation / Sex	Cohort 1A males				Cohort 1A females				
Dose [mg/kg/day]	0	6.4	21.5	64.4	0	6.7	22.8	68.1	
Animals per dose	10	10	10	10	10	10	10	10	
	Day 90				Day 90				
T ₄ [nmol/L]									
mean	75.20	77.55	75.01	79.44	61.64	68.82	60.44	55.44	
SD	18.42	11.72	17.01	10.67	12.23	9.04	15.91	5.64	
Δ%		3.1	-0.3	5.6		11.6	-1.9	-10.1	
TSH [μg/L]									
mean	6.60	7.42	8.46	9.28	5.32	5.45	5.20	6.76	
SD	1.32	1.26	4.42	2.37	0.91	0.97	0.77	3.21	
Δ%		12.4	28.2	40.6		2.4	-2.3	27.1	

^a Data obtained from Tables IB 36-IB 37 on pages 371-372 of the study report.

6. Postmortem results

- Organ weights:** Organ weight determinations in Cohort 1A animals revealed increased absolute and relative liver weights in mid and high dose males and females (Table 20), which is similar to F0 parental animals. While kidney and thyroid weights were statistically

significant for high dose males, these were not considered adverse since the difference was <20%. There was no effect on organ weights in Cohort 1B parental animals, which was mainly restricted to reproductive organs.

Table 20: Mean (±SD) organ weight for Cohort 1A (rearing animals) ^a								
Dose (mg/kg/day)	0	6.4	21.5	64.4	0	6.7	22.8	68.1
	Males				Females			
Terminal body weight (g)	318.5±25.1	321.0±27.3	322.9±31.7	331.7±20.9	201.3±16.0	199.5±12.7	196.3±9.9	194.7±10.4
Absolute								
Kidney	2.16±0.27	2.2±0.20	2.27±0.23	2.39±0.22** (↓10.6%)	1.46±0.13	1.45±0.10	1.46±0.10	1.46±0.10
Liver	8.1±0.74	8.33±0.97	8.87±1.28* (↑9.5%)	10.62±1.27** (↑31.1%)	5.08±0.42	5.19±0.36	5.51±0.31** (↑8.5%)	6.78±0.82** (↑33.5%)
Thyroid glands	21.4±3.95	22.5±3.27	24.1±4.97	25.4±5.06** (↑18.7%)	16.15±3.01	17.45±4.29	17.4±3.91	17.8±2.40 (↑10%)
Relative to Body Weight								
Kidney	0.68±0.05	0.69±0.05	0.70±0.05	0.72±0.05** (↑5.9%)	0.73±0.04	0.73±0.04	0.74±0.03	0.75±0.05
Liver	2.54±0.12	2.59±0.15	2.74±0.19** (↑7.9%)	3.2±0.24** (↑26.0%)	2.52±0.11	2.60±0.11* (↑3.2%)	2.81±0.11** (↑11.5%)	3.48±0.3** (↑38.1%)
Thyroid glands	0.007±0.001	0.007±0.001	0.007±0.001	0.008±0.002** (↑14.3%)	0.008±0.001	0.009±0.002	0.009±0.002	0.009±0.001 (↑12.5%)

^a Data obtained from Tables IC 16/51 – IC 23/51 on pages 396-403 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.

b. Pathology

- i. **Macroscopic examination:** There were no treatment-related findings observed at necropsy in Cohort 1A rats.
- ii. **Microscopic examination:** Histopathological examination of Cohort 1A animals identified the same target organs, i.e. the liver and thyroid as in F0 parental animals (Table 21). The minimal (Grade 1) centrilobular hepatocyte hypertrophy in two mid dose males was not accompanied by adverse liver associated clinical chemistry changes. However, the hypertrophy observed in the high dose males and females was accompanied by increase in liver weight as well as a drastic increase in GGT and an increase in incidence and severity of multinucleated hepatocytes. Thus, taken together, the liver effects observed at the high dose were considered toxicologically adverse. The increased incidence and severity of thyroid follicular cell hypertrophy/hyperplasia and altered colloid observed at the high dose for males and females was considered treatment-related and adverse.

Trifludimoxazin/080800

Table 21: Selected histopathological findings of Cohort 1A (rearing) rats administered BAS 850 H								
Dose level [mg/kg/day]	Cohort 1A males				Cohort 1A females			
	0	6.4	21.5	64.4	0	6.7	22.8	68.1
Animals examined	20	20	20	20	20	20	20	20
Liver								
- Multinucleated hepatocytes				6				
Minimal				3				
Slight				3				
Average				1.5 [#]				
- Hypertrophy. centrilobular			2	9				
Minimal			2	4				
Slight				5				
Average			1.0	1.6				
- Hypertrophy. diffuse				5				17
Minimal				2				7
Slight				3				9
Moderate								1
Average				1.6				1.6
- Pigment. hepatocytes				8				12
Minimal				8				12
Average				1.0				1.0
Thyroid glands								
- Hypertrophy/hyperplasia, follicular epithelium			1	14			4	13
Minimal			1	4			3	7
Slight				10			1	5
Moderate								1
Average			1.0	1.7			1.3	1.5
- Altered colloid	10	6	15	19	5	1	-	16
Minimal	5	6	12	2	5	1		9
Slight	4		3	4				2
Moderate	1			7				4
Marked				6				1
Average	[1.6]	[1.0]	[1.2]	[2.9]	[1.0]	[1.0]		[1.8]

[#] = mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding

^a Data obtained from Table IC 29/51 on page 409 of the study report.

D. DEVELOPMENTAL NEUROTOXICITY – COHORTS 2A AND 2B

1. Behavioral testing (Cohort 2A only)

- a. Acoustic startle: There were no effects of treatment on any acoustic startle parameter examined (Table 22).

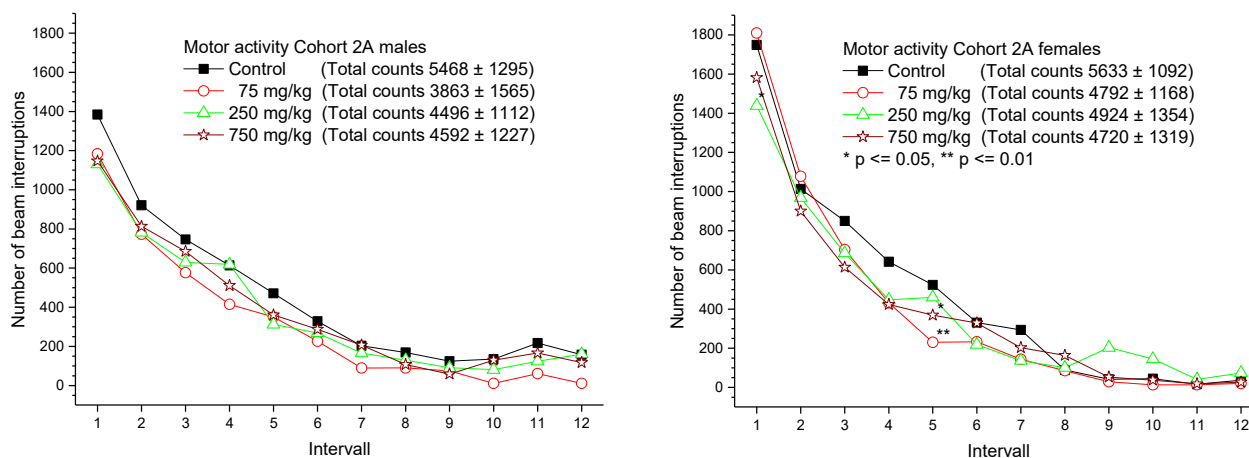
Table 22: Auditory Startle Response (ASR) of Cohort 2A animals									
Sex & Parameter		Males				Females			
Dose	[mg/kg/day]	0	6.4	21.5	64.4	0	6.7	22.8	68.1
Animals per dose		9	10	10	9	10	10	10	10
- Maximal amplitude [mV]	(mean)	292.1	282.5	241.1	242.3	295.9	263.0	279.8	309.2
	(SD)	120.1	94.6	56.5	34.0	159.0	89.0	51.6	82.5
- Latency [msec]	(mean)	23.4	20.9	22.0	20.1	23.5	22.9	25.0	23.0
	(SD)	5.2	3.2	2.6	1.8	6.7	3.5	4.9	4.9

- b. **Functional observational battery:** Neither home cage or open field observations nor sensorimotor test and reflexes performed at PND 72 revealed any treatment-related findings (Table 23). Likewise, measurement of quantitative parameters did not reveal and indicated of an effect of treatment. The determination of quantitative FOB parameters (number of rearings and depositions of feces, forelimb and hindlimb grip strength and food splay) did not result in significant differences between controls and treated groups.

Table 23: Quantitative FOB parameters of Cohort 2A animals									
Sex & Parameter		Males				Females			
Dose	[ppm]	0	75	250	750	0	75	250	750
Animals per dose		9	10	10	9	10	10	10	10
- Feces deposition [n]	(mean)	0	0	0	0	0	0	0	0
	(SD)	0	0	0	0	0	0	0	0
- Rearing [n]	(mean)	9	8	7	8	13	14	14	12
	(SD)	3	3	2	2	2	9	3	3
- Grip strength forelimb [Newton]	(mean)	9.0	9.3	9.2	9.2	8.0	8.3	8.2	8.3
	(SD)	0.9	0.7	1.2	0.9	1.0	0.7	1.0	0.9
- Grip Strength Hindlimb [Newton]	(mean)	4.5	4.9	4.7	5.0	4.1	4.1	3.7	4.0
	(SD)	0.6	0.6	0.7	0.5	0.3	0.4	0.4	0.6
- Foot splay [cm]	(mean)	10.0	9.9	9.8	9.6	9.5	9.2	9.3	9.4
	(SD)	0.6	0.4	0.4	0.8	0.4	0.5	0.6	0.6

- c. **Motor activity:** There were no treatment-related effect observed on the overall number of ambulation and fine movements (Figure 2). Occasional reductions ($p \leq 0.05$) in both observations were observed in females but did not show a dose response or were consistent over time.

Figure 2. Motor activity of Cohort 2A animals



2. **Brain weights:** There were no effects of treatment on brain weights in either Cohort 2A or 2B.
3. **Brain morphometry:** Brain morphometry data are presented in Table 24. The thickness of the right frontal cortex and the width of the left hemisphere of the Nucleus caudatus were significantly smaller in high dose males. However, the corresponding left and right structures did not display comparable changes. Thus, these changes were considered incidental and unrelated to treatment. The corpus callosum was also statistically significant smaller; however, the percent change between the control and the high dose (13.3%) was lower than the coefficient of variation (15.4%). Thus, this change is within the variability of the measurement and not considered adverse.

Table 24: Selected morphometric findings of the brain of Cohort 2A rearing animals (adults) (Mean±SD) ^a					
Sex		Males		Females	
Dose	mg/kg/d	0	64.4	0	68.1
F ₁ cohort 2A rearing animals. PND 77					
Frontal cortex					
- right hemisphere	[mm] Δ%	1.76±0.06	1.64±0.11* -6.8	1.64±0.09	1.68±0.11 2.4
- left hemisphere	[mm] Δ%	1.74±0.08	1.68±0.09 -3.4	1.66±0.08	1.65±0.14 -0.6
Nucleus caudatus, width					
- right hemisphere	[mm] Δ%	3.57±0.29	3.45±0.15 -3.4	3.28±0.19	3.42±0.22 4.3
- left hemisphere	[mm] Δ%	3.43±0.16	3.13±0.24** -8.7	3.18±0.22	3.29±0.17 3.5
Corpus callosum					
- width	[mm] Δ%	0.75±0.15	0.65±0.10* -13.3	0.73±0.12	0.70±0.10 -4.1

* p ≤ 0.05; ** p ≤ 0.01 (Wilcoxon tests, two sided)

^a Data obtained from Table ID-9/16 on page 440 of the study report.

4. **Neurohistopathology:** Neurohistopathology of Cohort 2A (adult) and Cohort 2B (weanling) animals did not reveal any treatment-related findings.

E. DEVELOPMENTAL IMMUNOTOXICITY (COHORT 3)

1. **Mortality and clinical signs**

- a. **Mortality:** There were no test substance-related or spontaneous mortalities in any dose group.
 - b. **Clinical signs:** No clinical signs or changes of general behavior, which may be attributed to the test substance, were detected in any of the male and female animals in any of the groups.
2. **Body weights, body weights gains, and food consumption:** There were no effects on body weights, body weight gains, or food consumption.

3. **Postmortem results**

- a. **Organ weights:** Organ weight determinations in Cohort 3 were restricted to the spleen and thymus. No statistically or biologically significant differences were noted in treated groups, while absolute and relative spleen and thymus weights were significantly decreased in the positive control groups (4.5 mg/kg bw/day cyclophosphamide).
- b. **Pathology**
 - i. **Macroscopic examination:** Few macroscopic findings were observed in Cohort 3 animals; these consisted of a reduced eye size in a mid-dose male, enlarged kidneys in a high-dose female and thymus discoloration of a low dose males. These single effects were determined to not be dose-related and therefore, not considered adverse. No macroscopic findings were recorded in positive control Cohort 3 animals.
 - ii. **Microscopic examination:** No histopathological examinations were performed in Cohort 3 animals.
4. **Immunotoxicological parameters:** No treatment-related or statistically significant differences of SRBC IgM titers were noted in groups any treated Cohort 3 group. In contrast, the positive control cyclophosphamide (CPA) resulted in a significant decrease of SRBC IgM titers.

III. DISCUSSION AND CONCLUSIONS

- A. **INVESTIGATORS CONCLUSIONS:** Under the conditions of the present modified extended 1-generation reproduction toxicity study the NOAEL for general, systemic toxicity is 250 ppm (equivalent to 28 and 25 mg/kg bw/d for males and females, respectively) for the F0 and F1 parental as well as adolescent animals, based on evidence for liver toxicity, as well as corresponding effects on clinical-pathological parameters, which were observed at the LOAEL of 750 ppm (equivalent to 85 and 76 mg/kg bw/d for males and females, respectively).

The NOAEL for fertility and reproductive performance for the F0 and F1 parental rats is 750 ppm (equivalent to 85 and 76 mg/kg bw/d for males and females, respectively), the highest dose tested. The slightly increased and treatment-related incidence of abnormal sperm in high dose (750 ppm) Cohort 1A males did not result in impaired fertility of F1 (=Cohort 1B) parental animals, which sired their female mating partners at the same age at which the slightly increased incidence of abnormal sperm was observed in high dose Cohort 1A males.

The NOAEL for developmental toxicity in the F1 and F2 progeny is 750 ppm (equivalent to 85 and 76 mg/kg bw/d for males and females, respectively).

The NOAEL for developmental neurotoxicity for the F1 progeny is 750 ppm (equivalent to 85 and 76 mg/kg bw/d for males and females, respectively), the highest dose tested.

The NOAEL for developmental immunotoxicity for the F1 progeny is 750 ppm (equivalent to 85 and 76 mg/kg bw/d for males and females, respectively), the highest dose tested.

B. REVIEWER COMMENTS:

There were no effects of treatment observed on mortality, body weights, body weight gains, or food consumption during pre-mating, gestation, or lactation, hematology, clinical chemistry, or urinalysis in parental animals. The liver and thyroid were identified as target organs. Liver effects observed at the high dose included increased organ weight, increased GGT, and increased incidence and severity of multinucleated hepatocytes. Thyroid effects observed at the mid- and high-dose included increased incidence and severity of follicular cell hypertrophy/hyperplasia and altered colloid.

The LOAEL for parental toxicity 250 ppm (equivalent to 21.5 and 22.8 mg/kg bw/d for males and females, respectively) based on increased incidence and severity of follicular cell hypertrophy/hyperplasia and altered colloid in the thyroid in males. The NOAEL is 75 ppm (equivalent to 6.4 and 6.7 mg/kg bw/d for males and females, respectively).

The reproductive performance of F0 and F1 (=Cohort 1B) male animals was affected by treatment with an increase in abnormal sperm observed at the high dose. No effects on the estrus cycle, mating behavior and fertility and the ability of females to deliver and rear their offspring was noted in both generations. Sporadically statistically significant differences of parameters were noted, but these were either not dose dependent, not observed in the other generation and generally within the historical control range.

The LOAEL for fertility and reproductive performance for the F0 and F1 parental male rats 750 ppm (equivalent to 64.4 mg/kg bw/d for males) based on an increase in abnormal sperm. The NOAEL is 250 ppm (equivalent to 21.5 mg/kg/d for males).

The number, survival, body weight development and sex ratio of pups was not affected by treatment. Sexual maturation of males and females was comparable between all groups. No increase in the incidence of pup necropsy observations was noted with the exception of an increased incidence of dilated renal pelvises at the high dose of 750 ppm. However, dilated renal pelvises are a common finding in rats of the strain used. In the affected pups no compression of the parenchyma surrounding the renal pelvis was observed, which would have been an indication for an obstructed urogenital tract. The most likely underlying mechanism of renal pelvis dilation is the transient inhibition of renal growth, particularly regarding the length of the renal papilla during late gestation. Literature indicates that this type of renal pelvis dilation often is reversible postnatally. Therefore, this finding is considered to be of no toxicological relevance.

The LOAEL for offspring toxicity cannot be established. The NOAEL is 750 ppm (equivalent to 64.4 and 68.1 mg/kg bw/d for males and females, respectively), the highest dose tested.

No neurotoxicological effects of treatment were recorded as neither the Functional Observation Battery (FOB) investigations (including home cage and open field observation, sensory motor and reflex tests and motor activity determination) in Cohort 2A animals at PND 72 nor the brain weight and/or brain morphology investigations at PND 22 (Cohort 2B)

and PND 77 (Cohort 2A) revealed any treatment-related changes. Furthermore, neither auditory startle response habituation at PND 24 nor neurohistopathological examination of Cohort 2A animals revealed effects related to treatment.

The LOAEL for developmental neurotoxicity for the F1 progeny cannot be established. The NOAEL is 750 ppm (equivalent to 64.4 and 68.1 mg/kg bw/d for males and females, respectively), the highest dose tested.

Investigation of the humoral, T-cell dependent immune response in sheep red blood cell (SRBC) immunized Cohort 3 rats did not reveal a change of Anti SRBC-IgM titers in blood six days after immunization. In contrast, Anti SRBC-IgM titers the Cyclophosphamide treated positive control animals were significantly decreased. The determination of splenic lymphocyte subpopulations (B and T lymphocytes, CD4+ and CD8+ lymphocytes and Natural killer cells) in Cohort 1A animals did not indicate an effect on cellular immune response.

The LOAEL for developmental immunotoxicity for the F1 progeny cannot be established. The NOAEL is 750 ppm (equivalent to 64.4 and 68.1 mg/kg bw/d for males and females, respectively), the highest dose tested.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OCSPP No guideline; OECD 443) for an extended one-generation reproduction toxicity study in the rat.

- C. **STUDY DEFICIENCIES:** There were minor deficiencies noted. First, the manner of defining a runt for the litters was not according to guideline. The guideline states that animals with body weights more than 2 standard deviations below the mean pup weight of the respective litter should not be included. However, the study report defines a runt as $\frac{3}{4}$ 25% below the mean body weight. Secondary, the time duration of the detailed clinical observations was not specified.

Appendix I. Range-finding study in Wistar rats administration via the diet for 4 weeks

CrI:WI (Han) male rats (10 per group) were exposed to 0, 750, 1000, or 1250 ppm (equivalent to mean concentration of 66, 86, 109 mg/kg/d, respectively) of BAS for 4 weeks. Mortality, clinical signs, food consumption, drinking water consumption, body weight, gross pathology, and sperm parameters (sperm motility, morphology, head count [cauda epididymidis], and head count [testis]) were examined. Summary of results are shown in Table 1. Specific sperm parameters are shown in Table 2. Incidence of gross lesions are shown in Table 3.

Table 1. Summary of Results				
	0 ppm	750 ppm	1000 ppm	1250 ppm
Clinical Observations	Findings: NAD FC: NAD BW: NAD BWC: NAD	Findings: NAD FC: NAD BW: NAD BWC: NAD	Findings: Ataxia (slight; days 7-11; 10/10) FC: NAD BW: NAD BWC: NAD	Findings: Ataxia (slight; days 7-11; 10/10) FC: NAD BW: NAD BWC: NAD
Clinical pathology	NAD	Increased incidence of abnormal sperm Decreased sperm motility	Increased incidence of abnormal sperm Decreased sperm motility	Increased incidence of abnormal sperm Decreased sperm motility
Pathology	NAD	Kidney cyst discoloration (1/10) Epididymides focus (1/10)	Liver discoloration (1/10)	Kidney cyst discoloration (1/10) Liver discoloration (4/10)

FC: Food consumption

BW: Body weight

BWC: Body weight change

NAD: No abnormalities detected

Table 2. Sperm parameters

		0 / M 0 ppm	1 / M 750 ppm	2 / M 1000 ppm	3 / M 1250 ppm
MOTILE_C [%] day 28	Mean	74 x-	68 *	50 **	37 **
	S.d.	18	11	27	26
	N	10	10	9	10
	Median	78	72	57	42
TS/gT [Mio/g] day 28	Mean	110 x-	113	98	103
	S.d.	19	21	12	15
	N	10	10	10	10
	Median	106	123	93	101
TS/gC [Mio/g] day 28	Mean	184 x-	145	164	114
	S.d.	77	58	89	56
	N	10	10	10	10
	Median	159	147	136	98
ABNORMAL6_C [%; Cut off 6%] day 28	Mean	12.4 x+	22.8 **	40.5 **	48.0 **
	S.d.	7.2	7.3	29.6	26.0
	N	10	10	10	10
	Median	12.2	22.5	27.2	41.0

* p≤0.05

** p≤0.01

X: Group excluded from statistics

Table 3. Incidence of gross lesions				
Dose (ppm)	0	750	1000	1250
Animals in group	10	10	10	10
Number abnormalities	0	2	1	5
Epididymides	--	--	--	--
Focus	--	1	--	--
Kidneys	--	--	--	--
Cyst	--	1	--	--
Discoloration	--	--	--	1
Liver	--	--	--	--
Discoloration	--	--	1	4